THE DEVELOPMENT OF THE BALANCER IN AMBLYSTOMA TIGRINUM

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TWO PLATES (FOURTEEN FIGURES)

INTRODUCTION

The occurrence of a balancer in a race of Amblystoma tigrinum has been previously reported (Nicholas, '24). The developmental details, however, remain to be described. They afford an interesting study from the standpoint of tissue potency.

The presence of balancers in various forms has long been a point of unstable observation, and it was not until the present year that Harrison ('24) collected the literature and brought the problem up to date. Since he has given a comprehensive historical survey of the whole question, the reader is referred to his paper for details which are not necessary here.

The observations here presented confirm the supposition of van Bambeke ('80), who thought that balancers were present in A. tigrinum at an early stage in rudimentary form, and also the opinion of Maurer ('88) that balancers might occur sporadically in axolotl embryos. Maurer actually observed the presence of this structure in one embryo out of sixty.

Since many investigators are engaged in researches upon Amblystoma, it is of some importance to clear up some of these small details which have been designated as characteristic in classifying the different species. Up to the present, the current opinion has been that if an amblystoma embryo

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possessed balancers, it was not A. tigrinum. Although undoubtedly there have been similar observations to those recorded here, there is no way of collecting these scattered observations and establishing either the truth or falsity of the common opinion.

The present study shows that balancers occasionally are present in larvae of this form. It has its particular value in that the eggs upon which the observations were made were laid in the laboratory from a known stock secured supposedly from North Dakota.[1] The adults are unmistakably A. tigrinum.

EXTERNAL DEVELOPMENT

The balancer of this race of A. tigrinum is first visible externally at stage 35. At this time the balancer region is marked by an excessive aggregation of pigment just inferior and slightly posterior to the eye. Following this (stage 36) there is a slight bulging of the ectoderm with a subsequent evagination at stage 37 (fig. 1). Growth continues up to stage 39, as is shown in figures 1 to 6, at which time the balancers can be recognized distinctly by their projection from the body wall and also by the direction of their growth. They present a definite prolongation of tissue with the proximal portion or base slightly posterior to the eye. The balancer extends ventrally and posteriorly and is clearly demarked from the region posterior to it by a definite groove, the hyomandibular groove. This cleft between the balancer and the lateral surface of the head is clearly shown in section (fig. 13). The distal portion of the balancer is rounded and extends posteriorly across this groove, reaching its maximum development at stage 38–39 (figs. 5 and 6).

Balancers were observed in 82 per cent of these animals. A larger percentage could probably have been secured if a

[1] It has been hard to fix definitely the locality from which this stock was secured. The collectors originally informed the writer that the stock was secured from Wisconsin, and has been so reported (Nicholas, '24). Material sent to Professor Harrison by the same collectors at the same time was reported to have come from North Dakota. In recent correspondence with the collectors, the same source has been reported.

greater number of cases could have been studied and the earlier stages of development been subjected to closer scrutiny for excessive pigmentation in this region.

The following tabulation is based upon animals which showed a definite balancer swelling and the subsequent growth

TABLE 1

Balancer development

STAGE	29	34	37	38-39	40-41
Balancer bud	Not visible on surface	Pigmentation and swelling	Definite balancer bud	Maximum development	Resorption

TABLE 2

Occurrence of balancer

NUMBER OF ANIMALS	STAGE	BALANCER PRESENT	BALANCER ABSENT
5	30	?	
4	34	4	
14	35	12	2[1]
19	37	18	1
24	38	22	

[1] These animals showed histological differentiation in the balancer region on section.

TABLE 3

Balancer measurements

ANIMAL	STAGE	TOTAL BODY LENGTH	BASE OF BALANCER		LENGTH OF BALANCER	
			Left	Right	Left	Right
		mm.	*mm.*	*mm.*	*mm.*	*mm.*
B 14	38+	9.5	.28	.30	.16	.12
B 8	39	10.	.40	.38	.43	.30
B 1	38	9.6	.30	.29	.24	.18
B 4	38	8.2	Swelling only—double layer of cells			

of the organ. The observations were made upon the living embryos which were followed throughout development to learn the fate of the balancers.

During stage 39, sometimes slightly earlier, one or both balancers may be lost by resorption. This is not a sudden

change; development ceases, the balancer region is flattened by the growth of the surrounding parts of the head and the balancer is completely obliterated. In unoperated animals no balancers were observed after the completion of stage 39. The appearance of the balancer region at stage 40 is shown in figure 8.

INTERNAL DEVELOPMENT

The development of the balancer bud in A. punctatum is characterized by an early stage of pigment aggregation followed by an ectodermal thickening caused by changes in the ectodermal cells of this region. The ectodermal covering is double layered and the internal layer becomes columnar in form. The same sequence is found in A. tigrinum, although its formation occurs slightly later. There is no apparent structural difference between the two species during the initial development of the balancer.

A comparison of balancer development in the slightly later stages shows practically the same sequence of events for the two species except for one definite particular. In punctatum embryos the ground-substance underlying the balancer gives rise to a definite balancer basement membrane at a very early stage. Its formation in this particular region precedes its general formation as a structure underlying the ectoderm.

In the tigrinum embryos this is not the case. The general basement membrane is formed first in the anterior region of the embryo, its formation proceeding posteriorly and ventrally. At the latest stage at which balancers have been found in tigrinum embryos, there has been no formation of a basement membrane at the level of the balancer. Never, in any case, is a definitive balancer basement membrane formed. This is true in both the normal and experimental series (cf. figs. 11 and 12, also figs. 13 and 14).

EXPERIMENTAL

After the observation of balancers in these forms, an attempt was made to prolong the existence of this structure by experimental methods. The experiments were exceedingly simple and are reported here in condensed form.

Stimulation of the developing balancer bud by dividing it or by pricking the bud itself or the tissues in proximity to it gave the following results: Division or stimulation anterior to the balancer bud increased the span of existence for one complete stage (stage 39–40, fig. 7). Stimulation superior to the bud produced a slight increase in the length of time during which the bud was present. Posterior and inferior stimulation of the tissue produced negative results.

Extirpation of the ectoderm comprising the bud resulted in healing without subsequent regeneration. This occurs also in A. punctatum. Harrison secured regeneration in six of eighteen cases, twelve failed to regenerate. In a series of thirty tigrinum embryos in which bilateral extirpation of the balancer bud was performed, I have not been able to observe a single case of regeneration. The tissues surrounding the balancer swelling, therefore, do not possess the capacity for regeneration.

Transplantation experiments were not performed. Harrison, however, carried out a heteroplastic transplantation in which tigrinum ectoderm from this region was transplanted to punctatum embryos, but no balancer developed. The reciprocal operation, however, i.e., punctatum balancer on tigrinum embryo, gave a perfectly normal balancer with a definite balancer basement membrane.

It appears, therefore, that the balancer swelling in these tigrinum embryos is composed of ectoderm which is capable of evagination. This tissue is capable of evaginating, per se, and is not dependent for this property upon the underlying tissues. The formation of the balancer basement membrane is dependent upon the influence of the balancer ectoderm, and it is not absent because of a difference in the visible materials composing the underlying tissues. The balancer ectoderm of these tigrinum embryos does not exert the formative influence necessary for balancer membrane formation.

ADDITIONAL OBSERVATIONS

Since the above observations were made, it has been possible for the author through the kindness of Professor Harrison[2] to make observations upon two other races of embryos derived from different stock. An examination of several hundred preserved specimens, supposedly of North Dakota stock, laid at the Osborn Zoological Laboratory at Yale, gave negative findings so far as a definite balancer was concerned. There is, however, an area of heavy pigmentation and slight swelling in the region occupied in the other forms by the balancer rudiment. In cross-section this pigmented area shows a double cell layer with cells of the columnar type in the internal layer. Observations upon larvae secured from animals shipped to Professor Harrison from Berlin showed but three animals out of seventy in which there was a thickened region of ectoderm. Two of these appear in figures 9 and 10. Other individuals showed an aggregation of pigment in this region.

This leads of course to the conclusion that the pigmented region represents the area destined in some individuals to form a balancer. The mechanism capable of causing balancer formation happens to be deficient in some forms and, therefore, balancer development is restricted and rudimentary. This deficiency in potency is shown by the inability of the evaginating ectoderm to cause the formation of a balancer basement membrane.

DISCUSSION

Larvae of Amblystoma tigrinum possess an area of ectoderm which, under certain conditions, in some races, is capable of forming a balancer. This is indicated, first, by the excessive pigmentation of the given area and, second, by the evagination and outgrowth of the ectoderm to form a rudimentary balancer. This is not of functional importance, for development ceases and resorption is complete before

[2] I am greatly indebted to the authorities at the Osborn Zoölogical Laboratory, Yale University, for the courtesy of the laboratory during the summer of 1924.

the organs could be of any possible functional significance. In Amblystoma punctatum the balancer passes through the same initial stage of development, but continues its growth and becomes functional (Harrison, '24).

There are two main factors aside from the species difference in the development of the balancer in these two forms. The first factor is that of time of development. The balancer begins to develop at an earlier stage in punctatum, stage 33, while in tigrinum the earliest indications are later in appearance, stage 35–36. This is hardly a factor in the early resorption of the balancer. The tigrinum limb develops considerably later than the punctatum, but apparently is controlled in a similar fashion. Certainly, it is of the same functional significance in both species.

The second factor is the formative relationship between the balancer ectoderm and the underlying tissue. The visible materials composing the balancer regions in the two species is identical during the initial stage of balancer formation. Later the balancer region of A. punctatum changes markedly. The balancer basement membrane is formed in advance of the general body basement membrane. In contrast to this, no basement membrane is formed in connection with the balancer of the present tigrinum series. The general basement membrane does not reach this region until long after the resorption of the balancer. This is true even when development is prolonged by experimental methods. At this stage (40–41) the general basement membrane extends only to the lower edge of the lens.

The balancer forming tissue in A. tigrinum is apparently incapable of causing the formation of the balancer basement membrane. Correlated with this deficient formative potency, there is complete absence of ability to regenerate as shown by the extirpation experiments.

The absence of a functional balancer in larvae of A. tigrinum is due, then, not to an absence of visible balancer forming material, but to the inability of the balancer forming tissues to bring about the formative condition in the under-

lying tissues whereby the formation of the balancer basement membrane is effected. In the absence of this membrane, the tissues with prospective balancer potency are unable to survive as balancers.

SUMMARY

1. A perfectly definite although rudimentary balancer has been observed in the larvae of Amblystoma tigrinum.

2. The embryos upon which this study is based were reared from eggs laid in the laboratory and are therefore of known parentage.

3. The development of this rudimentary balancer is identical in the early stages with the balancer development of A. punctatum.

4. Examination of later stages of development shows that the basement membrane which is formed very early in the balancer region of A. punctatum is lacking in these tigrinum embryos.

5. By simple experimental methods the duration of existence of the tigrinum balancer has been prolonged. Stimulation of the tissues just anterior and superior to the balancer bud gives the greatest degree of prolongation. The balancer bud will not be regenerated after simple extirpation.

6. The deficient potency of the ectoderm composing the balancer swelling is responsible for the rudimentary condition of the balancers in these larvae. This is shown by its inability to form a balancer basement membrane and also by its inability to regenerate.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 to 6 Successive stages of Amblystoma tigrinum to show the development of the balancer. × 7. 1, stage 37—; 2, stage 37+; 3, stage 38; 4, stage 38+; stage 5, 39—; stage 6, 39.

7 The balancer bud was stimulated by pricking the region just anterior to the swelling at stage 36. The balancer of the unoperated side has been completely resorbed, while the operated balancer is just past the peak of its growth, The delay in resorption effected by stimulation amounts to one full stage of development. Stage 40. × 7.

8 Stage 40, slightly older than the preceding. Both balancers have been resorbed.

9 and 10 Amblystoma tigrinum, Berlin stock. The animal shown in figure 9 marks the peak of balancer development in this series of observations. Figure 10 shows a little better development than the average for the series—the majority of the animals showed excessive pigmentation only. × 7.

1
2
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4
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8
7
10

PLATE 2

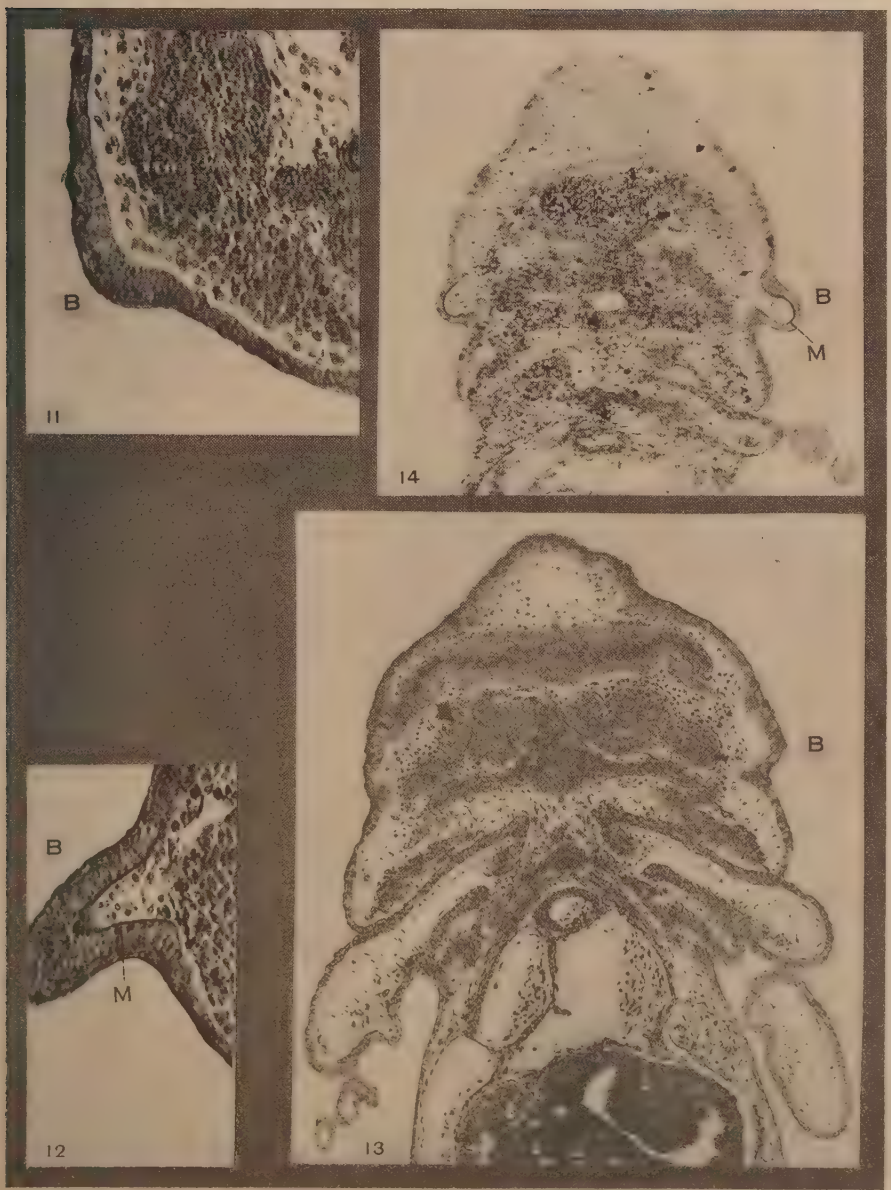
EXPLANATION OF FIGURES

11 Cross-section through tigrinum balancer, stage 38. × 100. Note the double-layered ectoderm and the absence of basement membrane.

12 Cross-section through punctatum balancer, stage 37. The black line just internal to the balancer is the balancer basement membrane. Compare with preceding figure. × 100.

13 Frontal section through tigrinum balancer. × 50. Note the groove which separates the projecting portion of the balancer from the head and the absence of basement membrane.

14 Frontal section through the balancers of A. punctatum. Compare the basement membrane with preceding figure. × 50. *B*, balancer; *M*, balancer basement membrane.


B
11
B
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THE SO-CALLED HIBERNATING GLAND IN MAMMALS: A FORM OF ADIPOSE TISSUE[1]

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TWO PLATES (TWELVE FIGURES)

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INTRODUCTION AND HISTORY

The so-called hibernating gland is a curious gland-like adipose tissue which is present in all hibernating mammals—with one or two exceptions—and in some, at least, of the non-hibernating mammals, such as the albino rat, tame mouse, and possibly man. The term 'hibernating gland' is retained throughout this article for the sake of convenience, although, as will be pointed out later, this tissue hardly deserves such a distinctive designation. It is known in the literature under a number of different names (Rasmussen, '23), and although there has been a tendency in recent years to consider it as a ductless gland of considerable importance, its function is still in doubt.

[1] The writer wishes to acknowledge his indebtedness to Prof. S. H. Gage and Dr. B. F. Kingsbury for suggestions and generous assistance throughout the course of the investigation.

Rasmussen ('23), in a recent article on the hibernating gland of the woodchuck, which contained a review of the literature on the subject, divides the history of the interpretation of the hibernating gland from a functional point of view into three or four periods. The first period extended from the time of its discovery by Velsch in 1670 to 1817, during which it was generally mistaken for a part of the thymus, but still a ductless gland, usually grouped as of the hemopoietic series. Since 1863 and by a few writers even before this, it has been considered as a modified form of adipose tissue serving merely as a storage for foodstuffs. Finally the interpretation of its endocrine character has been revived several times, especially since 1902. Rasmussen points out, however, that it would be unwise to group this brown multilocular adipose tissue with the endocrine organs, since there is no evidence pointing at all strongly to its internal secretory character, and no research has gone extensively into its phylogeny and its physiological significance to give data of any reliance.

The typical hibernating gland is light brown in color and its glandular appearance is partly due to the loose, fibrous connective-tissue septa dividing it into irregular lobules. It is separated from the surrounding structures by a fibrous connective-tissue capsule and in places by ordinary adipose tissue, which also frequently extends between the lobules. A few nerve fibers and large blood vessels are found in the connective-tissue septa. The parenchyma is composed of densely packed polygonal cells containing a large amount of highly granular cytoplasm. The large spherical nucleus is usually central in position, unless it has been pushed to one side by one of the numerous fat-globules, which vary in size and number in the different cells. The intercellular spaces are abundantly supplied with numerous small blood vessels.

The name of the species in which the hibernating gland has been found, its location in the body, the authority therefor, etc., are found in the table originally compiled by Auerbach ('02), then slightly added to by Polimanti ('12),

and finally extended and modified by Rasmussen ('23). The regions most often mentioned in this table as containing hibernating gland tissue are in the order of their importance: the thoracic, cervical, axillary, scapular, renal, and the inguinal.

It is not the purpose here to review the literature on the subject since this has been so admirably done by Rasmussen ('23). The primary purpose of this paper is to point out certain more or less definite changes that occur in the appearance of the hibernating gland as a result of growth, altered nutrition, etc., which may perhaps aid in a better interpretation of its nature. Cramer ('20) regards this glandular type of adipose tissue as functionally closely related to the endocrine glands, especially the thyroid and the adrenal. The significance of these observations rests upon the generally accepted assumption that functional activity is correlated with structural changes. However, no attempt has been made here to associate these structural changes with any corresponding changes in functional activity. It has also been noted in this investigation that while in some species this brown adipose tissue persists throughout life, in others it is transformed comparatively early in the life of the individual into ordinary adipose tissue.

MATERIAL AND METHODS

The material used in this investigation was mostly taken from white rats, some from cats, and several specimens from a monkey. All the animals were killed by illuminating gas, except the monkey, which was chloroformed. The tissues were fixed immediately after death.

Quite a number of fixers were used, but the ones that gave the best results were Benda's, Zenker's, Zenker's with reduced acetic, and Regaud's potassium bichromate and neutral formalin solution. The stains used were copper hematoxylin, iron hematoxylin, anilin acid fuchsin, and methyl green. Material fixed in Regaud's potassium bichromate and neutral formalin by injection, followed by

potassium bichromate, and then stained with anilin acid fuchsin and methyl green, gave the clearest pictures, particularly of the mitochondria present. Schridde preparations in which the tissues were mordanted in a bichromate solution before immersion in 2 per cent osmic acid had the advantage of producing stainable compounds of cell proteins and lipoids which were not subsequently dissolved out by alcohol and a clearing oil.

OBSERVATIONS

Rat

The rat is not a true hibernating animal, but possesses a hibernating gland comparable in location and appearance to the hibernating gland of typical hibernating animals. The availability of the albino rat and the fact that it is rapidly becoming the standard laboratory animal make it exceedingly valuable in experimental and other investigations.

Position and relation of the lobes. The hibernating gland of the rat may be found in well-nourished animals in all the regions mentioned by Rasmussen ('23) in his table, but it is sometimes almost or entirely absent in the inguinal region in animals which have been on a subnormal diet. The largest lobes are on the back between the scapulae, where there are two large, well-defined, gland-like lobes of a reddish-brown color with small extensions cephalad and laterally between the muscles. There is another pair of large lobes beneath the vertebral column in the region of the kidneys. These lobes may partially or almost entirely surround the adrenals and some of the sympathetic ganglia. Cramer ('20) says that they also more or less surround the ovaries in the female. There are other small lobes in the cervical, axillary, and thoracic regions. In the latter it is frequently found between the lobules of the thymus.

Microscopic structures. All the lobes are identical in histological appearance except that the cervical, axillary, and inguinal frequently contain lymph nodes and, as I have said before, the perirenal sometimes encloses sympathetic ganglia.

As all of the lobes are practically identical, I have largely confined my observations to material from the interscapular region, where it is most abundant and readily obtained. The lobes are separated into irregular lobules throughout their entire mass, and a small amount of connective tissue is present between and within the lobules. A varying amount of ordinary adipose tissue may also be present within and between the lobules (fig. 2). This tissue is very vascular and capillaries practically surround each cell, as is admirably illustrated by the specimens prepared by injection of the fixer as advocated by Cowdry ('18, figs. 1, 2, 3, and 4).

The large polygonal cells are from 25 μ to 40 μ in diameter. The cell body is laden with many comparatively small fat and lipoid droplets, and the nearly spherical nucleus is nearly always central in position, and though it may occasionally be pushed to one side, it is never flattened. The nucleus is about 7 μ in diameter and contains a dense, coarse, irregular chromatin network with one or two fairly large acidophilic nucleoli. In addition to the fat-droplets, the cytoplasm is crowded with small spherical granules (mitochondria?) of a fairly uniform size (½ μ, figs. 1 and 2), although there are a few larger ones which approach the size of the smaller fat-droplets.

Changes in the hibernating gland as a result of feeding on a fat-rich diet. Rasmussen ('23) says that there is very little evidence in the woodchuck—and very little, if any, in any other animal—of a transformation of hibernating gland cells to ordinary fat-cells, although the latter may be mixed with the former where the two tissues are in contact. Auerbach ('02), however, reported what he thought was a distinct transformation of hibernating gland cells into ordinary fat-cells. My observations tend to confirm those of Auerbach that there is a distinct transformation and this is especially noticeable in the rat when fed on a fat-rich diet (figs. 6 and 7).

The appearance of the hibernating gland is noticeably altered by feeding an animal on a fat-rich diet for a period of only one week. Neither the cytosomes nor the nuclei are

apparently increased in size, but the fat-globules themselves are distinctly larger, and hence there are fewer of them (fig. 5). There is also a decided increase in the number of unilocular fat-cells within and between the lobules. This increase occurs to a much greater extent at the periphery of the lobule, but the degree of transformation varies in the different lobules. This increase in the number of unilocular fat-cells is much more evident in a rat fed on a fat-rich diet for a longer period (one to two months), but even then a very considerable amount of the adipose tissue remains in the multilocular condition. In some lobules about one-half the cells are of the unilocular type, while other lobules are composed almost entirely of unilocular cells with only a comparatively few multilocular cells remaining near the center of the lobule (fig. 6). The process of this transformation of multilocular into unilocular fat-cells can readily be followed. The globules of fat become larger and larger and fuse together until finally only one large globule of fat is present in the cell, and the nucleus is flattened against the cell wall. That this transformation is accompanied by a distinct increase in the total amount of fat within the cell is evident, as many of the unilocular cells are two or three times the diameter of the multilocular cells.

The question also arises as to whether age as well as diet might not be an important factor in this transformation. The previous material had been obtained from young adult rats, but material was also obtained from an old rat (three to four years) which, although it had been on a normal diet, was in an almost emaciated condition. Under the microscope this material showed a very large proportion of unilocular cells and some of the lobules had been almost entirely transformed into unilocular fat-cells (fig. 8). From this observation, I have no doubt that there is a steady increase in unilocular cells and a corresponding decrease in multilocular cells as the animal increases in age.

Changes in the hibernating gland as a result of hunger. When a rat is allowed water, but no food for a period of

four or five days, the ordinary adipose tissue almost entirely disappears. The hibernating gland tissue is more persistent, but becomes reduced in size and assumes a somewhat darker color. Many of the fat-globules are reduced in size, and there seems to be fewer globules in the cell, while the cytoplasm seems to be much more granular.

If food is withheld from the rat for four or five days, and it is then allowed all the fatty foods it will eat for a period of about seven hours before being sacrificed, there is a comparatively rapid deposit of fat again in the tissue of the hibernating gland. This can be macroscopically demonstrated by feeding the rat on fatty foods which have been colored with sudan III. The sudan III remains with the fatty acids with which it is absorbed and is not transferred to the fat which was laid down previously (Gage and Fish, '24). When this tissue is examined microscopically, it is noticed that in general the droplets of fat in the cells are smaller than those of a rat fed on a normal diet, and there are very few unilocular cells present in the lobules. However, the cells are not very noticeably affected in either size or appearance.

In a rat from which food was withheld for ten days, the fat-globules were much smaller and of a much more uniform size. The cells were reduced in diameter approximately one-fourth of their former size, but the nuclei apparently remained unchanged (figs. 3 and 4).

With further emaciation, the nuclei as well as the cell body diminish in size. The cytoplasm stained darker, probably due to the larger percentage of proteins in the cytoplasm, and the few small fat-droplets which remained in the cells were not dissolved by the fixer (Regaud's). These fat-droplets (?) stained very much like the large fuchsinophile granules, which will be described later.

Presence and behavior of mitochondria. In addition to the many fat-globules of varying size present in the cells of the hibernating gland, one can by special methods of fixation and staining readily observe numerous small, round granules, which possess the staining reactions of mitochondria. These

mitochondria are small, spherical, slightly refractive bodies of a fairly uniform shape and size (about $\frac{1}{2}$ μ in diameter), although there are a few larger ones which approach the smaller fat-globules in size. The larger fuchsinophile granules (mitochondria?) stain even more readily than the smaller mitochondria. They are relatively more abundant and larger in the cells of the hibernating gland of the rat when fed on a fat-rich diet than in the normal hibernating gland (fig. 5).

The possibility of the transformation of the mitochondria into fat has been widely discussed by many authors, and the majority agree that they may be transformed into fat-droplets. Whether this transformation is real or merely an imbibition of fatty material into the interior of the mitochondrium has not been satisfactorily determined. Cowdry ('18) may be referred to for a discussion of the relation of mitochondria to lipoid.

During this investigation I have noticed that it is quite easy to distinguish typical mitochondria from typical lipoid, but it is almost impossible to tell whether the large fuchsinophile granules are true mitochondria or true lipoid or represent a stage in the transformation. A reciprocal relationship between the relative amounts of mitochondria and lipoid was noted in all the tissues studied. In those cells—usually found in the younger animals—where there was a relatively small amount of lipoid, the cytoplasm contained a large number of mitochondria, while in those cells with a large amount of lipoid, there were comparatively few mitochondria.

In the cells of the hibernating gland of the rat deprived of food until death occurred, there were practically no vacuoles (dissolved fat) present, but the cells contained many large fuchsinophile granules (3 μ to 4 μ in diameter). These granules stained similarly to the mitochondria with anilin acid fuchsin, iron hematoxylin and copper hematoxylin after both Regaud's fixation and copper bichromate-sublimate-formalin fixation (Kingsbury, '12). This may have been due to the fact that the more labile lipoids had been used up

during the course of the fasting, leaving only the more stable lipoids with essentially the same staining reactions as the mitochondria.

Cat and monkey

Position and relation of the lobes. The general location of the lobes in the cat is practically the same as in the rat, except that they are not confined to any definite location, but can be found in any place—especially in young kittens—where adipose tissue is likely to be found in the adult cat. In addition to lobes in the interscapular, thoracic, cervical, perirenal, axillary, and inguinal regions, well-defined, characteristic lobes were found in the mesentery, and also subcutaneously in the abdominal and inguinal regions.

In the new-born kitten, the hibernating gland in the interscapular region was not as far advanced in development as in the other regions of the body, and to the naked eye the gland appeared as many small almost microscopic lobules of a reddish-brown color in the midst of a mass of embryonic mesenchymal tissue.

Microscopic structure. The hibernating gland tissue from the interscapular region of the new-born kitten microscopically presented the appearance of 'primary adipose tissue formation' as described by Hammar ('95). The cells were rounded or polygonal and very granular. Many were without any fat-vacuoles. They were grouped in lobules of varying size. Even at this early stage of adipose tissue formation, the very rich vascularity of the typical hibernating gland tissue was beginning to make its appearance, and the cells seemed to be grouping themselves in relation to particular blood vessels.

However, in the other regions, where the hibernating gland tissue is found, the cells were in well-defined lobes, and contained a comparatively large number of fat-vacuoles. One very noticeable difference between the cells of the hibernating gland of the kitten and those of the rat was the number and size of the fat-globules. While the cells of the hibernating

gland of the rat contained a number of small fat-globules, many of the cells of the kitten frequently contained only two or three fat-globules, and one of these was usually much larger than the rest, occupying the entire central portion of the cell, and shoving the nucleus to one side, but never flattening it.

In the hibernating gland tissue taken from a kitten a little over a day old, there was a very noticeable increase in the size of the cells, and also in the number of fat-globules in the cells. Although there were one or two large fat-globules in the majority of the cells, the number of smaller globules had materially increased. Many cells contained no conspicuously large globules, but contained many of a fairly uniform size, as in the hibernating gland of the rat (fig. 9).

At first glance, the hibernating gland of the kitten three weeks old very much resembled the appearance of the hibernating gland of the rat when fed on a ration rich in fat. However, the number of unilocular cells was not as large as mere casual observation might suggest, on account of the fact that while a large number of the cells throughout the lobule were occupied by one or more large fat-globules, there were in the majority of the cells quite a number of very small peripheral fat-droplets surrounding the large central globules. But at the edge of the lobules, just as in the hibernating gland of the rat fed on a ration rich in fat, there were large numbers of unilocular fat-cells (fig. 10).

The lobes, however, were not all in the same stage of development, as those in the inguinal region were very largely composed of cells containing only small fat-globules; hence they present a very compact appearance in contrast to the lobules from the other regions of the body.

In a young adult cat the only region where the hibernating gland tissue could be definitely located with the naked eye was in the perirenal region, and even there the lobes were very much lighter in color than the same tissue in the rat, probably due to the fact that the number of unilocular cells exceeded those of the multilocular type. In some lobules the

transformation was almost entirely complete and only a few multilocular cells remained to indicate that the entire lobules had consisted of multilocular adipose tissue cells (fig. 11).

Hibernating gland tissue was located in the lateral and ventral cervical region, the thoracic region near the thymus, and the axillae of a young, almost adult monkey (Macacus rhesus). It was not noticed in the inguinal region, and no attempt was made to locate it in the interscapular or perirenal regions. Microscopically it very much resembled the tissue of the kitten a little over a day old and the rat fed on a fat-rich diet, except that the cells in general were slightly larger and seemed to contain fewer very small fat-droplets (fig. 12). Several places were noted on the periphery of the lobules where a transformation of the multilocular cells into unilocular cells was evident.

NATURE OF THE SO-CALLED HIBERNATING GLAND

From the literature on the development of the so-called hibernating gland, it seems clear that it develops in much the same manner as ordinary adipose tissue. The cells arise from branched connective-tissue cells, which become round or polygonal before any fat is deposited in them. However, the cells are more granular than ordinary adipose tissue cells and the droplets of fat fail to unite into one large globule. It is evident, then, that the so-called hibernating gland is essentially a form of adipose tissue which retains its embryonic characteristics for a more or less indefinite length of time. It has been shown in this investigation that there is a distinct intergradation of this brown multilocular adipose tissue into ordinary white adipose tissue. Under favorable conditions, there is probably a slow, but constant transformation of the multilocular into the unilocular type. The so-called hibernating gland is undoubtedly widely present in both hibernating and non-hibernating mammals, although its presence has not been reported in many of the latter. It is my opinion that it scarcely deserves a distinctive designation, as it is probably only a more or less persistent form of embryonic adipose tissue.

SUMMARY AND CONCLUSIONS

1. The hibernating gland of the rat is persistent throughout life, but some of the cells are transformed into ordinary adipose tissue cells.

2. This transformation can be hastened and considerably increased by feeding the animal on a ration rich in fat.

3. A similar but somewhat less extensive transformation occurs in the gland as a result of increase in age.

4. The mitochondria found in the cells of the hibernating gland gradually enlarge, and are apparently transformed into fat-droplets.

5. When the rat is deprived of food, the cells of the hibernating gland lose a large percentage of their fat and are much diminished in size.

6. The nuclei to all appearances remain practically unchanged by the altered conditions of nutrition and growth.

7. The hibernating gland of the cat is not persistent, and is gradually transformed into ordinary adipose tissue. This transformation is practically complete by the time the animal is fully grown.

8. Hibernating gland tissue was located in the cervical, thoracic, and axillary regions of an immature monkey.

9. The so-called hibernating gland resembles embryonic adipose tissue. It intergrades with ordinary adipose tissue, and under favorable conditions is transformed into it. It is widely present in mammals, but hardly deserves a distinctive designation as it is probably only a form of embryonic adipose tissue.

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A more complete and comprehensive bibliography will be found in the above article by Rasmussen.

PLATE 1

EXPLANATION OF FIGURES

1 Hibernating gland from the interscapular region of a normal young adult rat to show the typical appearance of the cells under normal conditions. Photograph retouched to better show the cell outlines. Regaud's solution injected; section .5 μ; stained with acid fuchsin and methyl green. $\times$ 380.

2 Same section as in figure 1, but taken near the periphery of the lobule to show the presence of unilocular fat-cells. Photograph slightly retouched. $\times$ 380.

3 Hibernating gland (interscapular) of a rat deprived of food for ten days. The capillaries are devoid of red blood cells and somewhat distended due to injection of the fixer. Compare the size of the cells with those shown in figure 1. Regaud's fixation; section 5 μ; stained with acid fuchsin and methyl green. $\times$ 380.

4 Drawing outlined with camera lucida of the portion of the enclosed area shown in figure 3. $\times$ 950.

5 Hibernating gland (interscapular) of a rat fed on a diet rich in fat for a period of one week. Notice that both the cells and the individual fat-globules are larger than those shown in figure 1. Regaud's fixation; section 5 μ; stained with acid fuchsin and methyl green. $\times$ 380.

6 Hibernating gland (interscapular) of a rat fed on a ration rich in fat for a period of about two months. This photograph was taken from the center of the lobule and shows the few multilocular cells which have not been transformed into unilocular fat-cells. Regaud's fixation; section 5 μ; stained with acid fuchsin and methyl green. $\times$ 380.

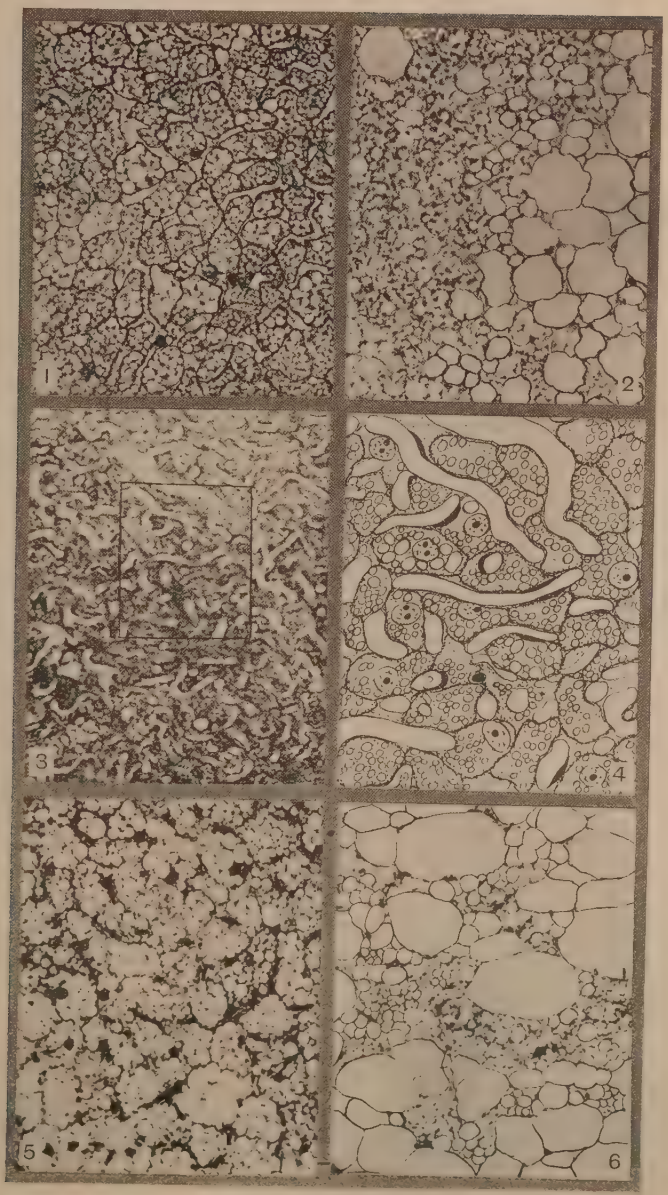
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PLATE 2

EXPLANATION OF FIGURES

7 Hibernating gland (interscapular) of a rat fed on a ration rich in fat for a period of about two months. Notice the intergradation of the multilocular into the unilocular adipose tissue. Absolute alcohol fixation; section 5 μ; stained with hematoxylin and orange G. $\times$ 95.

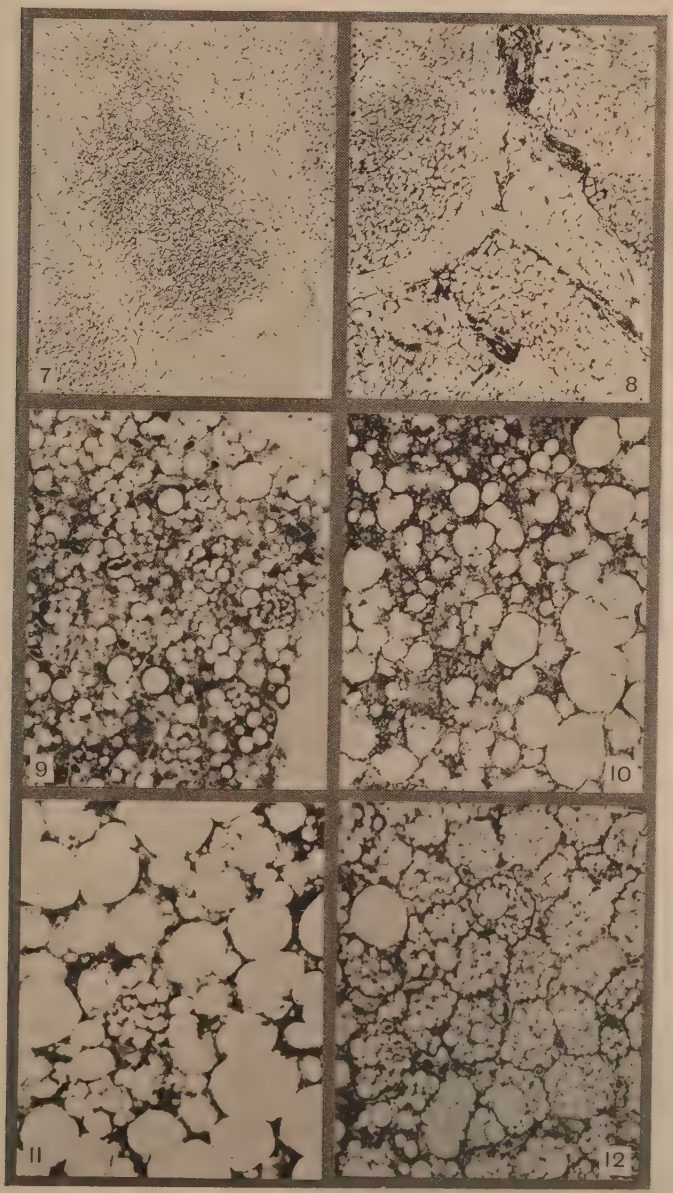
8 Hibernating gland (interscapular) of an old rat in an almost emaciated condition. Compare with figure 7 as to the comparative amounts of multilocular and unilocular adipose tissue. Chrome sublimate fixation; section 5 μ; stained with iron hematoxylin. $\times$ 95.

9 Hibernating gland (interscapular) of a kitten slightly over one day old. Notice that this tissue very much resembles that shown in figure 5. Regaud's fixation; section 5 μ; stained with acid fuchsin and methyl green. $\times$ 380.

10 Hibernating gland (interscapular) of a kitten three weeks old. Notice that many of the smaller globules have fused together to form large globules. Regaud's fixation; section 5 μ; stained with acid fuchsin and methyl green. $\times$ 380.

11 Hibernating gland (perirenal) of a young adult cat. Practically all of the cells are of the unilocular type and only a few multilocular cells remain and these are scattered here and there throughout the lobules. Regaud's fixation; section 5 μ; stained with acid fuchsin and methyl green. $\times$ 380.

12 Hibernating gland (axillary) of an immature monkey. Regaud's fixation; section 5 μ; stained with iron hematoxylin. $\times$ 380.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OBSERVATIONS ON THE LYMPHATIC NODULE, PARTICULARLY WITH REFERENCE TO HISTOLOGICAL CHANGES ENCOUNTERED IN SENESCENCE

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INTRODUCTION

Without attempting to review here fully the literature of the field, it is sufficient to state that in spite of innumerable researches, the opinions of modern workers are still at variance in regard to the interpretation of dense lymphoid tissue. A recent contribution to our knowledge of the histogenesis of lymphoid tissue was furnished by Latta ('21), who worked on the intestinal tonsils of the rabbit and who opened up a rather new aspect of the subject by declaring that the so-called 'germinal centers' found in lymph nodules were not primarily areas of proliferation, but centers of degeneration. His further work ('22) on the spleen emphasizes the same interpretation, which is diametrically opposed to the orthodox view, proposed by Fleming ('85), and since held by practically all modern workers, who ascribe to the lighter staining portion of the nodule the significance of lymphocyte production, and regard these regions as areas of proliferative activity, hence true 'germinal centers.'

At this time, then, the interpretation of the lymphoid nodule is perhaps more puzzling than it has ever been, and the present work, though undertaken with the purpose of gathering any data whatever which might have a bearing on unsettled questions pertaining to lymphoid tissue, was particularly stimulated by a desire to elucidate the germinal center controversy. The investigation was carried on under

the direction of Prof. B. F. Kingsbury, to whom I would here make grateful acknowledgment for much helpful advice as well as for generous provision of working facilities.

It was decided to confine the study to lymphoid tissue occurring at a definite point in the body, and for this purpose the caecum was selected. Both rats and cats were employed, both being easily procured, but due to the more compact localization of lymphoid tissue in the rat caecum, this animal was used almost exclusively in the development of the problem, the cat, and in one case rabbit, being referred to only for purposes of comparison.

It is well known that the amount of lymphoid tissue encountered in an animal body bears some relation to the age of the individual, and statement of this fact is made in standard texts (cf. also Settles, '20). Examination of the material in hand revealed the fact that there were certain evident modifications in the lymphoid tissue of the caecum which were to be correlated with the advancing age of the animal. Such modifications were: reduction in total area occupied by lymphoid tissue, smaller size of individual nodules, relative obscurity of the germinal centers, etc. It was thought that a careful study of the histological changes involved in this regressive process might be employed as a means of approach hitherto neglected in the study of lymphoid tissue problems. The tendency of previous workers has been to emphasize 'histogenesis,' but there seems no reason why the tracing of the fate of this tissue should not prove as important as the study of its origin.

Literature bearing intimately upon regressive changes associated with the disappearance of lymphatic tissue is not especially abundant. Jolly ('14), in a study of the effects of starvation upon lymphoid tissue in rabbits and puppies, found that all localizations of lymphoid tissue were thereby more or less reduced. Starvation effected a practically complete involution of the thymus and also affected to a somewhat less extent the tissues of the 'blood-lymph' group (spleen and bone-marrow). The 'lympholymphatic' group (lymph

nodes) were least affected but nevertheless showed marked regressive transformation. Jolly makes special mention of the fact that "in the appendix the disturbances are sometimes considerable." He observed rather carefully the histological changes in the lymph nodes and describes conditions strikingly similar to those encountered in the present investigation, and in this case due to advancing age. The same author ('15), in an extensive study of the bursa Fabricii of birds, produced experimental involution of that curious organ, both by starvation and by use of x-rays. The involution due to starvation was not definitive, for it was possible by restoring the proper diet to cause the bursa to reappear in a size proportionate to the age of the bird. Like the thymus, the bursa Fabricii undergoes complete involution normally fairly early in life. Jolly refers to this organ as a 'cloacal thymus,' and points out the similarity between these two transitory lympho-epithelial organs.

The work of Settles ('20), already referred to, and more recently of Lefholz ('23) has shown the stimulating effect of particularly rich diet upon lymphoid tissue in all parts of the body—a diet rich in fat and at the same time high in calories being especially effective in increasing the amount of lymphoid tissue above the normal. Evidently, whatever other factors may be concerned, general nutritive conditions have much to do with lymphoid-tissue fluctuation, and the lowered metabolism of old age may be held at least partly responsible for the normal diminution in amount of lymphoid tissue in later life.

MATERIAL AND TECHNIQUE

In all, tissue from eighteen rats, six cats, and one rabbit was examined, the age of each being at least approximately known in all cases. Four very old rats (nos. 16, 17, 18, 19 of this paper), whose ages were known to the day, furnished clear examples of more or less advanced regressive change due to senility.

Sections were cut 8 and 10 μ and serially mounted. These were variously stained most satisfactorily by the use of alcoholic eosin and alkaline methylene blue. Wright's blood stain gave practically the same results. Zenker's fluid, picro-aceto-formol, and Helly's Zenker-formol were used in fixing, the last most frequently.

OBSERVATIONS

We may best begin with a description of the conditions found in the young adult and later compare with these the conditions encountered in later life.

In the rat the caecum is quite large, uniformly tapering, and lies against the ventral abdominal wall nearly opposite the midline. Its tip is curved toward the left, which side, the mesenteric, is concave. On the dorsal side (inward surface) at a point less than 6 mm. from the tip and about 90° removed from the mesentery, we find the only dense lymphoid tissue possessed by the rat's caecum below the ileocaecal valve. Here we find a patch of nodules, never in my observation exceeding twenty-five, and in outline roughly that of a blunt spearhead, with the tip pointing downward.

In the cat the situation is not so easily summed up, since the lymphoid-tissue area is not definitely limited. In proportion to the size of the animal, the caecum is much smaller than in the rat. The lymphoid tissue is abundant from near the tip to a point about half-way between the apex and the entrance of the ileum. There is some evidence of greater condensation on the side away from the ventral body wall, but the constancy of such a condition is doubtful.

The rabbit, with its extremely long appendix of almost uniform diameter, exhibits more lymphoid tissue in this region than either of the others, it being possible to demonstrate lymphatic nodules in almost any part of the wall.

The microscopic picture in the young adult rat presumably demonstrates the lymphoid tissue at the height of its development, or possibly at the very beginning of its decline. All surrounding tissue seems to have given way before the

growth of the nodules, which are large, closely compacted, and taken together make a tumorous bulge, filling nearly one-third of the lumen of the caecum in certain individuals. In this region tunica propria, muscularis mucosae, and submucosa are practically obliterated, a very narrow strip of submucosa separating the base of the nodules from the circular muscle layer. The muscularis seems to make some attempt to surround each nodule, but even this continuity is frequently interrupted. What portion of the tunica propria may have escaped obliteration is so completely infiltrated with lymphocytes as to give the appearance of what we might call 'diffuse lymphoid tissue.' The surface epithelium, which elsewhere is associated with conspicuous glands, is, over each nodule, either stretched out and non-glandular or is interrupted. The latter condition usually prevails, but whether naturally or by reason of faulty technique, it is not always easy to say. Nevertheless, at such apertures I have frequently seen evidence of lymphocytes making their way into the intestinal lumen. I may also add at this point that, so far as my observations are concerned, I have never seen lymphocytes penetrating the epithelium and entering the lumen at points where the epithelium was entire. They pass into the epithelium, it is true, and lodge there, but their further migration into the intestinal lumen is very doubtful.

The epithelium immediately above a nodule exhibits very few, if any, goblet cells, whereas in the more glandular epithelium elsewhere such cells are abundant and conspicuous. Such a condition was noted by Latta ('21) in the rabbit. The significance of this condition is at present obscure.

Passing to the nodule itself, we find a peripheral region made up largely of more or less densely packed, basic staining cells, among which the small lymphocyte is the predominant cell type. On the very surface of the nodule we find lymphatic vessels, and lymphocytes appear to be migrating into these in large numbers. Blood vessels, fairly abundant, many of considerable size, appear here and there in the peripheral region, and it is about these and about the

lymphatics that the condensation of lymphocytes is greatest. Migration of lymphocytes may be seen taking place into the blood vessels also, but not in such numbers as into the lymphatics, where, no doubt, the thinner walls of these vessels make penetration easier. There is a fairly uniform distribution of larger lymphoid cells, which, following Latta, we may group together under one name, 'lymphoid haemoblasts.' These are obscured by the greater number of small lymphocytes, however. Degenerative cell forms are rarely to be seen in the peripheral region, which is as we might expect considering the abundant vascular supply of this area. Mitotic figures may be observed occasionally, but when found seem to be always confined to an area slightly more diffuse than the surrounding region. Such areas are usually bounded by trabeculae in such a way as to protect them from the crowding of the many adjacent cells. Mitotic figures almost invariably occur in groups. Due to the condensation of cells in the periphery, the reticular tissue framework can here be demonstrated only with difficulty.

Turning our attention now to the so-called 'germinal center,' we find, as have others, that its lighter staining reaction is due principally to two things: first, a much more diffuse grouping of the cells, due largely to the fact that the cells here are predominantly of the lymphoid-haemoblast type, and, being larger, leave larger intercellular spaces, and, second, the presence of a considerable number of large, sometimes very large cells with more or less acid staining cytoplasm, a very variable nucleus, not always discernible, and with many cytoplasmic inclusions, variable in size and in staining reaction, which seem to represent ingested remnants of degenerate cell nuclei. Rarely two or three of these large cells are associated together. These 'acid staining macrophages' have been noticed by many workers. Flemming's ('85) tingible Körper, and the cells described by Downey and Weidenreich ('12), also by Hartman ('14), were regarded by Latta ('21) as identical, and I am inclined to agree with his interpretation. Each of these 'macrophages' (rarely

two or three associated) lies in a more or less open area devoid of cells, save for occasional forms which are apparently degenerating. This condition also adds to the general diffuse appearance of the 'germinal center,' taken as a whole. It is to be noted that these acid staining macrophages are apparently peculiar to the 'germinal centers.' I have never seen them in the peripheral region.

There are always a few of the small lymphocytes to be found in the 'germinal centers,' but in the better developed centers, especially, they are not abundant, and are rather uniformly scattered. The point of chief interest in the 'germinal center,' however, was the finding of mitotic figures, usually occurring in groups, but sometimes as isolated cells in process of division. This brings support to the orthodox view which regards the lymphocytes as originating by cell proliferation within the 'germinal centers.'

The blood vessels present are both smaller in size and fewer in number than those found in the periphery. The reticular tissue, though rarely very distinct, is much easier to demonstrate than in the peripheral region. The fairly conspicuous groups of degenerating cells tend to occur in the near vicinity of the acid staining macrophages, which, in their turn, are usually remote from any vascular supply. There is good evidence that the reticular tissue is here relatively more conspicuous, not alone because of the more diffuse association of the cells. It also appears to be here and there somewhat hypertrophied, and now and then there is evidence of reticular tissue cells rounding off, becoming detached, and taking on lymphoid form. This is in accordance with the work of Thiel and Downey ('21) on the "Development of the Mammalian Spleen." It is directly opposed to the opinion of Saxer ('96), who studied the development of the lymphatic nodes. Latta ('23) mentions the work of these authors, and tends to agree with Saxer in that he fails to find, in the spleen, convincing evidence of the transformation of reticular tissue cells into lymphocytes.

As for mitotic figures, I believe that they tend to be localized toward the periphery rather than in the very center. They almost always occur in close proximity to blood vessels, and in the only sections in which they occur throughout the field, blood vessels also are evident in the center of the nodule. Furthermore, a study of serial sections, with one exception, has shown that such a picture represents a tangential section through the 'germinal center.'

In the young adult rat, where the 'germinal center' appears to be at its maximum development, there is a sharp line of demarcation between it and the peripheral zone. Under other conditions, this line of demarcation is not so clear, and there is a considerable zone of intergradation, in which mitotic figures may usually be found, if they are to be seen anywhere. Thus one is sometimes at a loss to know where to assign a particular group of cells, and this may, in part, explain the differences of opinion as to where mitoses are found to occur.

Finally, passing without the nodule, we find almost always at certain points in the connective tissue adjacent to the lymphoid tissue area a fair abundance of eosinophiles, though not in such numbers as to be unusually conspicuous. In the areas where they seem most numerous there is not infrequently a slightly 'teased' appearance to the connective tissue. Such pictures are rare in young specimens, but become rather conspicuous in older tissue. I find good evidence of occasional transformation of connective-tissue cells into eosinophiles, not a few showing the eosinophilic granules, before becoming completely detached from the connective-tissue matrix. This is a point disputed by several workers, but the evidence seems fairly conclusive that we are here dealing with eosinophiles of connective-tissue origin.

In the young cat and in the young rabbit the well-developed nodules show, in general, a marked similarity to those just described for the young rat. One notices differences, however, in certain features pertaining to the 'germinal center.' In defense of Latta it may be said that, as a rule, it is indeed

difficult to find mitotic figures in the 'germinal center' of the rabbit. As though in correlation with this there seem to be a greater number of acid staining macrophages and relatively smaller number of blood vessels in the rabbit than we encounter in the 'germinal center' of the rat. In the cat, however, there is a fair abundance of blood vessels in this region, though the acid staining macrophages, as in the rabbit, tend to exceed in number those usually present in the rat. In the cat, also, mitotic figures are usually conspicuous in the 'germinal center,' and continue to be so, even in older specimens, where the general mass of lymphoid tissue is much reduced. It is difficult to explain these differences to our entire satisfaction, but it would surely appear that good nutritive conditions, as evidenced by the proximity of abundant vascular supply, is indeed the principal factor in stimulating proliferation of the lymphocytes.

For purposes of contrast, the most extreme case of regression, found in rat no. 18, may here be briefly summarized. This specimen was a female two years and eight months old. She was rather thin and scrawny, apparently not in good condition. Upon dissection of the caecum, in the area normally occupied by lymphoid tissue, examination revealed only a shrunken, wrinkled surface. The caecum wall at this point was certainly no thicker, and perhaps actually thinner than elsewhere.

Microscopic examination revealed in the submucosa a single condensation that might be said to represent a one-time nodule. It was noted that the epithelium immediately above this was glandular, at the same time crowded with lymphocytes, as was also the tunica propria between the glands. The nodule, if such it may be called, does not possess a 'germinal center.' A section through the center of this nodule shows it to be of uniform density, the small lymphocyte being the predominant cell form, with a few of the larger type scattered here and there. Eosinophiles are to be seen migrating into the nodule from the surrounding connective tissue, where they are abundant. Only occasionally can either mitotic or de-

generative figures be demonstrated. Migration of lymphocytes into blood and lymph vessels can be seen, also a few eosinophiles seem to be involved with the endothelium of the blood vessels, but whether migrating in or out it is impossible to say. None of the glands, already mentioned, appear to extend below the muscularis mucosae, which is everywhere entire. Of course, no acid staining macrophages are to be seen.

In this specimen, in particular, was the 'teased' appearance of the connective tissue especially marked. The impression given is that shiftings, due to collapse of a one-time distended region, are largely responsible for this distorted condition.

Between these two extremes may be ranged the conditions encountered in all the other material studied.

The lymphoid patch in the caecum of all younger animals approximated in appearance, more or less closely, that found in rat no. 5, aged six months, tissue from which formed the basis for the foregoing description of conditions in the young adult. Rats nos. 14, 17, 16, and 19, on the other hand, aged, respectively, two years six months, two years eleven months, two years eight months, and two years eight months, all exhibited more or less degeneration of the nodules. The extent of regressive change was progressively greater in the order listed, no. 14 exhibiting conditions closest to those found in the young rat, while in no. 19 the nodules were in an advanced state of degeneration. None of these animals, however, showed such complete involution of the nodules as was found in no. 18, already discussed.

The only animal examined in which conditions failed to correlate with the age was rat no. 13. The exact age was not available. The approximate age was given as 'nearly three years,' yet a great mass of lymphoid tissue was found to be present. But the caecum in this animal had been observed, upon removal, to be badly twisted and adherent, and microscopic examination soon revealed that the unlooked-for lymphoid patch was quite dissimilar in character from

that present in a young adult specimen. For reasons which will be discussed later, it was concluded that the picture presented here was due to pathological conditions, and that particular interest, therefore, attached to this apparent exception to the natural order of affairs.

Tissue from two very old cats was also examined, and in practically every way the observations confirm those made upon the rat. One cat was fifteen years old, the other twenty. In these animals there were only a few widely scattered nodules, distributed over the area where the young cat exhibits a mass of them, closely packed. The sections show mostly only loose-lying connective tissue, which exhibits the characteristic 'teased' appearance, already referred to. Several large, more or less circular 'empty spaces' in the connective tissue, containing usually a few lymphocytes each, may possibly be construed as representing the sites of recent nodules, the pressure of surrounding parts being not yet great enough to cause collapse of these areas.

DISCUSSION AND INTERPRETATION

We have seen how, in more or less direct correlation with the advancing age of the animal, there is reduction in the amount of lymphoid tissue present in the caecum. That this cannot be strictly correlated with the 'chronological' age of the animal is shown by the fact that rat no. 17, older than nos. 16, 18, 19, did not exhibit as pronounced regression as did they. Yet the general condition of no. 17 was excellent, whereas the others, especially no. 18, showed marked external indications of senility. The 'physiological' age, then, must be considered as the true index of internal conditions. The twenty-year-old cat, however, appeared to be in excellent physical condition for its age, nevertheless the lymphoid tissue had largely disappeared. The fact that this animal was a castrated male may partly explain its general sleek appearance, but it would nevertheless appear that we have here a condition enabling us to say, that, although good general condition may delay the 'passing' of lymphoid tissue,

this tissue is nevertheless among the first to go as the metabolism of the organism begins to decline.

We may trace the steps in the regressive process as follows: By a falling off in the production of lymphocytes, either by slower proliferation of those already present or by cessation of reticular tissue-cell transformation, the density of the nodule soon becomes less, due to the fact that as long as there is a supply of lymphocytes, they tend to migrate at once into the blood vessels and lymphatics. Thus, by a process in which migration out of the nodules exceeds supply, we arrive at a very diffuse condition throughout the nodules, most apparent of course in the 'germinal centers' where there are fewer blood vessels to attract the cells. Such condition obtains in rat no. 14, where apparently all nodules have undergone this change more or less simultaneously, and though somewhat flattened out, due to pressure of the intestinal content, they have not yet collapsed, since pressure has been temporarily reduced on every side.

The acid staining macrophages, which are known never to appear until the nodule is large enough to possess a 'germinal center,' begin to decline in numbers as soon as the regressive process starts. They evidently represent a type of degeneration peculiar to, and brought about by, germinal center conditions, and function principally as phagocytes, ingesting remnants of degenerated cells. The degeneration of cells is perhaps greatest when the nodule is at its maximum development, and may be due merely to faulty nutritive conditions, or possibly to the production of cells in greater numbers than can possibly migrate into the blood and lymph vessels. Such degeneration is, at least, most noticeable in areas remote from vascular supply, and in such areas there remains cellular debris, which is taken up by the macrophages, acting in the capacity of scavengers for the 'germinal center.' Having observed transitional forms, I am inclined to regard the macrophages as derived through transformation of lymphoid haemoblasts, as did Latta ('21).

In this connection the recent work of Maximow ('22, '23), on the culture of lymphoid tissue outside of the body, is especially enlightening, since he shows how reticulum cells as well as lymphocytes may transform into phagocytic macrophages. He also finds that larger lymphocytes may produce, progressively, leucoblasts (premyelocytes), young myelocytes, and finally mature myelocytes with characteristic granulation. Fibroblasts he finds to be definitive, but reticulum cells may take on a fibroblast-like form.

Most authors seem agreed that small lymphocytes may grow into large lymphocytes, and these, in turn, by cell division again produce smaller forms. Mitotic division of small lymphocytes is of course frequently observed also. It is especially to be noted that in the present investigation mitoses of lymphocytes were to be observed even up to the very last stages of nodular regression. Evidently, then, the principal factor in determining the shrinkage of the nodule is not the cessation of cell proliferation, but rather, it may be suggested, the exhaustion of the source of cell supply, presumably the reticular-tissue cells. Our work bears this out in that there is great difficulty in demonstrating transformation of these cells into lymphocytes in the older animals, while in the younger tissue it is at times rather easily seen. Maximow, it may be said, regards the transformation of reticulum cells into lymphocytes as occurring but rarely.

To continue with the fate of the nodule, we may say that as soon as its character becomes less dense, whatever stimulus maintained the zoning influence which caused the 'germinal center' to be differentiated ceases to be effective. There is, then, ample opportunity for all cells to migrate into blood vessels and lymphatics, hence this may account for the fact that degeneration of lymphocytes tends to become more rare, except in areas where nutrition is obviously poor. Since, in starvation, nutrition is necessarily unsatisfactory, it is not strange that Jolly ('14) saw much evidence of cell degeneration in advanced stages of lymphoid-tissue reduction, which had been brought about by intentionally imposing a rigorous fast.

The macrophages, then, which are to be correlated with the maximum development of the 'germinal center,' even as the 'germinal center' itself expresses the maximum development of the nodule, are no longer needed, and the transformation into acid staining cells no longer takes place. The fate of those already present is questionable. Many seem to be undergoing disintegration, but occasional retransformation does not seem impossible.

By this time connective tissue will have largely replaced the area previously occupied by the nodule. That the nodule diminishes faster than the replacement occurs is evidenced by the curiously diffuse and 'teased' appearance of the connective tissue found in the near vicinity at this time. Increased local production of eosinophiles seems to accompany this process normally, but why this should be is difficult of explanation. The origin of the granules within the cells is beyond doubt, however. Endogenous origin of eosinophilic granules has recently been demonstrated convincingly by Ringoen ('21), who was able to induce eosinophilia by bringing about anaphylactic conditions.

It seems to the writer that the eosinophilia is induced by increased toxic conditions within the caecum. This is perhaps to be linked up with one of the supposed functions of lymphocytes, wherein, by localizing in the intestinal epithelium, they may act as affixers of toxins. Bunting ('22), in a clear discussion, mentions this as possibly a very important function of these cels. Miss Florence ('22) suggests that the replacement of glands in the caecum by lymphatic nodules is essentially a protective process. If this be true, the removal of such tissue, even though a normal accompaniment of old age, would leave the region unprotected from toxic stimuli, and the production of eosinophilic granules may be, therefore, a direct response on the part of the connective-tissue cells to these conditions.

The question of the relation of glands to nodule formation is hardly a part of this paper, since we are primarily concerned with the conditions which obtain later. However, in some of the younger tissues examined, there were vestigial remains of glandular crypts within the nodules, showing

that gland degeneration had proceeded hand in hand with growth of the nodule. It has never been decided whether the growth of the nodule causes the destruction of the gland or whether degeneration of the gland stimulates nodule formation, but the relationship may perhaps be made the basis for a suggestion or two in connection with the character of the nodule. Miss Florence ('22), who worked on glands found in the caecum of several animals, makes the remark, "A germ center has not been seen in any of the follicles in which glands are present." Now it may be supposed that the 'germinal center' expresses the result of some zoning influence, such as remoteness from lymphatic vessels, and that this condition is brought about only when the ratio of mass of the nodule to its surface area reaches a certain point. Just why this should cause the large lymphocyte to predominate in the inner zone and the smaller lymphocyte in the outer we cannot attempt to say, but it seems highly probable that any condition which maintains a higher ratio of surface area to nodule mass will offset the tendency for the nodule to become thus differentiated. Thus we see that as long as the gland retains connection with the intestinal lumen such conditions are accomplished, and it is only when this continuity is interrupted that a 'germinal center' may be expected to appear.

In the last stages of nodule reduction (as in rat no. 18) the surface epithelium appears again more or less glandular, but this is not due, apparently, to any new gland formation; rather to the fact that the glands not immediately above any particular nodule, having been variously compressed and displaced during that nodule's period of maximum development, are now free to assume their normal position, more or less perpendicular to the inner surface of the caecum wall. Small, non-glandular portions of the epithelium, practically devoid of goblet cells, usually remain to mark the spots beneath which the individual nodules could once be found.

Rat no. 13, which exhibits a relatively large amount of lymphoid tissue for so old an animal, serves to illustrate

the fact that certain stimuli may cause resumption of lymphopoiesis, even in advanced age. In this animal, as already mentioned, the caecum was badly adherent. Pathological conditions were therefore at once suspected. The individual nodules were very tall, closely packed, and possessed well-developed 'germinal centers.' The connective tissue adjacent to the patch of lymphoid tissue was hypertrophied, imparting to the caecum wall more than usual thickness. Fortunately, the entire caecum was sectioned in this particular case, and careful examination revealed the presence of several eggs of an intestinal parasite, evidently a species of Cestode. It may safely be assumed that disturbances due to the presence of an intestinal parasite furnish adequate explanation for the unusual conditions here found. Strangely enough, eosinophiles were comparatively rare in the mucosa of this animal, but, following out our previous assumption, it may be that the hypertrophied lymphoid tissue is adequate protection against the toxic conditions, exaggerated though they be, and that there is at this point, therefore, little stimulus for granulopoiesis.

SUMMARY AND CONCLUSIONS

We have demonstrated that it is a normal occurrence in the rat for the lymphoid tissue in the caecum to undergo gradual reduction with advancing age, and that this may proceed to the point of almost complete obliteration. The conditions are to be correlated with the 'physiological' rather than the 'chronological' age of the animal, since an individual in good condition may sometimes possess more lymphoid tissue than individuals actually younger, but which are in an obviously senile state.

One may go so far as to say that reduction of lymphoid tissue by fasting may be essentially a demonstration of the same process normally taking place with advancing age.

In case of abnormal conditions, such as the presence of an intestinal parasite or other irritating factor, the process

may be reversed, and an abundance of lymphoid tissue persists even in an animal of advanced age.

The lymphocytes in the nodules apparently owe their primary origin to transformation of reticular-tissue cells. Mitosis of the lymphocytes occurs whenever and wherever nutritive conditions are favorable. Its occurrence in the 'germinal center' is limited by remoteness of most of the cells from the larger blood vessels and in the periphery by the closely packed condition of all the cells.

Degenerative processes seem to be initiated principally by faulty nutritive conditions, and are most abundant in the center of the nodule, where blood vessels are few and small.

Acid staining macrophages represent a form of degeneration peculiar to the 'germinal center,' and they appear to be an expression of the maximum development of the latter. These macrophages appear to be transformed lymphoid hemoblasts, which have grown in size and acquired phagocytic powers.

Due to the marked condensation of lymphocytes about both blood and lymph vessels, we are inclined to regard the periphery of the nodule as a zone of migration, rather than one of either developmental or degenerative change.

The 'germinal center,' however, is both a region of proliferation and cellular disintegration, the fate of any particular cell or group of cells being determined by the proximity of vascular supply. The 'germinal centers' are themselves expressions of the maximum development of the entire nodule, being as we know, never discernible in beginning nodules and inconspicuous in nodules that have undergone marked reduction. This seems to express a relation between the mass and the surface area of the nodule. The association of a degenerating gland with a nodule may temporarily alter this ratio and permit considerable enlargement without 'germinal center' formation.

The reduction in mass of the lymphoid tissue is usually accompanied by increase in number of eosinophiles in the connective tissue adjacent. These eosinophiles are transformed

connective-tissue cells which have developed endogenously the characteristic eosinophilic granules. From our knowledge of eosinophiles in general, it may be suggested that they appear as a result of increased toxic conditions at this point.

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FILING MICROSCOPIC SLIDES IN A CARD CATALOGUE

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THREE FIGURES

The lack of flexibility afforded by slide boxes as a place to store microscopic slides led the writer to work out a method by which slides could be kept in the ordinary 3-×-5-inch card files. This system has, of course, all of the possibilities of expansion and accessibility possessed by the card index and has the additional advantage over slide boxes in that all readings, notes, and drawings may be filed directly with the slides they refer to. This method of storing slides has been in use for five years and has proved perfectly satisfactory. During these years the writer's slide collection has several times been shipped for long distances by freight and express without damage to the slides.

To make the slide holders black cardboard is cut into 3-×-5-inch cards and an equal number of pieces 3-×-1½ inches. Medical wooden applicators (used as probes for nose and throat swabs) are cut to 5-inch and to 2¾-inch lengths. A strong paper or photo trimmer will cut thirty to fifty of these sticks at once. The sticks are then mounted on the 3-×-5-inch cards as shown by white lines in figure 1. It is most convenient to put the 5-inch stick on the bottom of the card first and to give the glue chance to set. To save constant measurement for the position of the short vertical sticks a piece of white cardboard was mounted on a thin board and ruled as shown (fig. 3). The black lines indicate the proper spacing of the short sticks. Pegs are placed as shown to

hold the cards square with the lines. The end sticks need no other guides than the edges of the card and the three middle sticks are placed, after being touched with glue, so as to coincide with the black lines. After the glue has set the 3-×-1½-inch cardboard strip is glued in place over the wooden strips, thus forming four pockets into which the slides slip easily (fig. 2).

The most convenient way to handle the wooden strips and the glue is to spread the latter in a thin layer on a sheet of glass and, holding a strip with forceps, touch it to the glue and apply it to the card. After the strips are in place and the adhesive dry the lower half of them is lightly touched to the layer of glue, sufficient adhering to hold the 3-×-1½-inch covers which are then added.

Black cardboard is used, as it does not show dirt and because the diamond-cut record figures on the slides can be easily read against the black background. Notations with white or colored pencils stand out clearly on the black surface. If the cards are needed for slides measuring 2 × 3 inches, the same cards but with only the middle partition stick left in place serve. In damp climates ordinary glue is not satisfactory. Either a water-proof glue must be used or thick shellac may be substituted. A system for recording microscopic readings, notes, etc., on 3-×-5-inch cards has already been published (Hance, '16).[1] The actual manufacture of these cards does not take long and the finished product costs only about one cent for three cards (storage capacity for twelve slides).

Recently Dr. Robert L. King, of the University of Pennsylvania, has had a paper manufacturer make for him a lightweight fiber envelop of the same dimensions as the above cards with spaces for slides marked off with wire staples sewn on a stitching machine. This is a very satisfactory form and, of course, relieves the user of the necessity of making the cards. They are somewhat more compact and

[1] Hance, Robert T.: A system for recording cytological material, slides and locations on the slides. Trans. Amer. Mic. Soc., vol. 35, pp. 57–60, 1916.

will probably prove more durable. The charge for making the first lot of these was about three cents apiece.

Eight of the home-made cards and twelve or thirteen of the fiber envelopes occupy 1 inch of space. Ninety to one hundred and forty cards, holding from 360 to 560 slides will fit

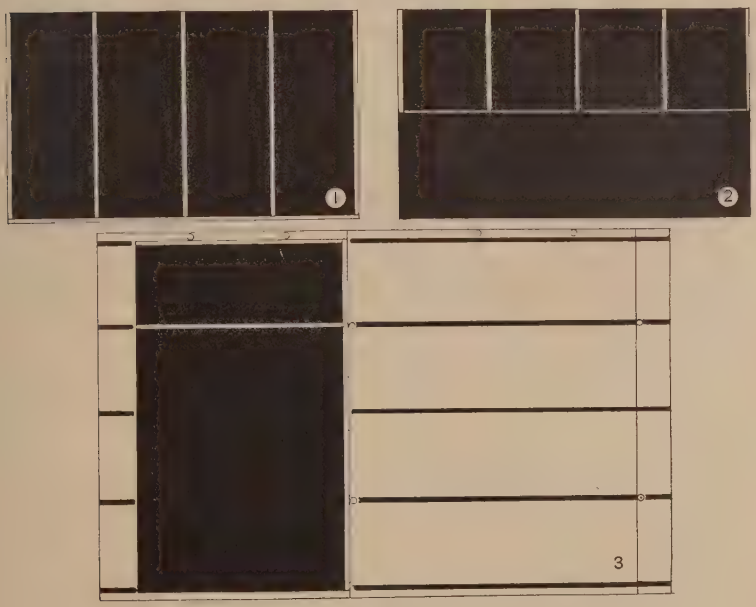

Fig. 1 Card with white lines indicating position of sticks.
Fig. 2 Completed card.
Fig. 3 Device for aiding in the making of the cards showing one card, partially finished, in place. Two cards are usually made at a time.

into the ordinary 3-×-5-inch cardboard filing case that is 11 inches long. Slide boxes of twenty-five-slide capacity to hold a similar number of slides would take up from 26 to 40 linear inches.

It is better to dry all slides thoroughly in an incubator or a warm room before putting them into the containers, so as to prevent the dammar or balsam from sticking to the card. If thin slides are used, there is, in the home-made card, enough

clearance between the top of the pocket and the cover to prevent adhesion if the card is allowed to lie flat until the balsam is dry. This is not true of the fiber cases. When small cover-glasses are used, a pocket formed by a strip narrower than the 1½-inch strip mentioned above would clear the area of fresh balsam in newly made slides, but even here it would be better to allow the container to lie flat until the balsam is dry.

Another method of utilizing for slides the same size catalogue drawer is in use in the Department of Biophysics of the Rockefeller Institute for Medical Research. The 5-inch-wide drawer is divided with narrow wooden partitions into four channels a trifle over 1 inch in width. Slides are placed vertically in these channels, with small strips of fiber board making the necessary separations. Individual slides lie against one another without damage.

CELL DISSECTION BY HAND

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TWO FIGURES

This paper describes a method for cutting cells requiring only a simple type of glass needle held in the hand. In this way many operations on eggs, protozoans, and other cells can be performed with a precision and control generally thought possible only with a special microdissection apparatus. It cannot take the place of the machine for the more critical types of cell dissection under high powers, but it places a considerable range of the microdissection technique at the disposal of investigators with a great saving of time.

In connection with studying the origin and nature of asters in enucleated eggs when artificially activated, it was necessary to secure within a short time a considerable number of enucleated Echinarachnius eggs—a demand which could not be met by a microdissection apparatus because of its complicated setup. It was in connection with using Chambers'[1] machine and making its needles, however, that this simpler technique was developed, and I wish to express my appreciation of Doctor Chambers' many suggestions and courtesies.

Hand dissection can be performed with a scalpel or an animal hair, but a glass needle has decided advantages over both. While dissection with metal has been used with success, it is frequently impractical. The knife edge, no matter how sharp, has microscopic nicks which frequently prevent a per-

[1] Chambers, R.: The microvivisection method, Biol. Bull., vol. 34, no. 2, Feb., 1918, pp. 121–136. New apparatus and methods for the dissection and injection of living cells, Anat. Rec., vol. 24, no. 1, Aug., 1922, pp. 1–19.

fectly clean separation of the cell, leaving little bridges of ectoplasm between the fragments. These bridges may tear and allow the surrounding medium to gain contact with the endoplasm, resulting in the bursting of the cell. In using the glass needle, the cutting is not done by the actual tip, but by the side of the finely rounded hair immediately behind the tip (fig. 1). This bears down on the cell pressing the upper surface against the lower surface, establishing complete contact between the two without tearing, before the cell is fragmented. Many cells if cut with a scalpel either burst immediately or soon cytolyze because of shock, but are unharmed if cut by glass. Another advantage is the thinness to which the cutting hair can be drawn, down to a diameter of 10 μ, which can hardly be duplicated in metal. Further, the glass hair does not obstruct the operator's view of the object; it does not interfere with the illumination; that part of the hair used in cutting can be kept entirely immersed so that there are no surface-film disturbances which interfere with vision and move about the objects; and there are none of the electrical phenomena associated with metal. In these points a glass needle is far superior to a scalpel, and makes possible more accurate dissections.

An animal hair has also been used as a cutting implement, but it has the same diameter and flexibility throughout and must be mounted in some manner. The needle here used is really a ready-mounted glass hair. Its chief advantage over animal hair lies in the fact that its diameter and flexibility range gradually from extreme fineness and pliability at the tip to relative coarseness and rigidity farther back, allowing various possibilities of manipulation by cutting with different regions.

The cutting is done on an ordinary microscope slide lying on the glass stage of a binocular dissecting microscope, so that the operation is seen through an uninverted image. The slide must be absolutely clean, so that the drop of water has not the least tendency to round up at the edges in order that it will be both wide and shallow. A large-sized drop is

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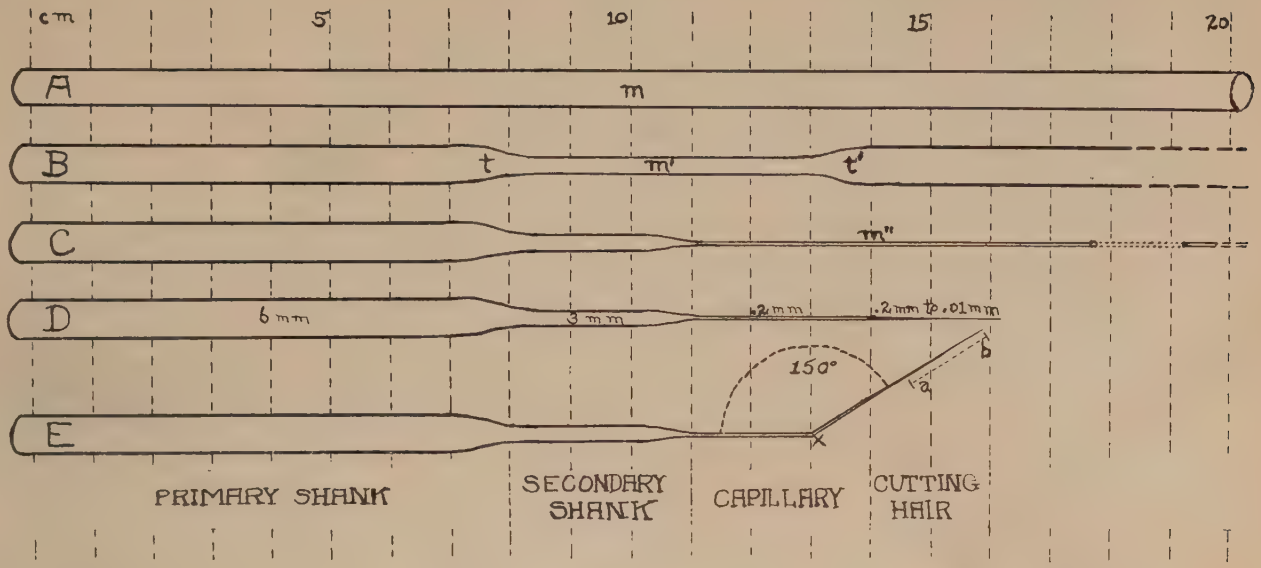

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Fig. 1 Diagrammatic view of cutting operation. That portion of the needle's cutting hair visible here is indicated by the line *a–b* in figure 2.

Fig. 2 Stages in needle making. A, Primary shank, 6 mm. tubing, for two needles, in which secondary shank tubing is to be drawn at *m*. B, Once-drawn tubing for two needles, in secondary shank stage, in which capillary tubing is to be drawn at *m'*. C, Twice-drawn tubing for one needle, in capillary stage, in which cutting hair is to be drawn at *m''*. Dotted lines indicate middle region of capillary which has been destroyed. Capillary of other needle shown at right. D, Thrice-drawn needle, with completed point, ready for bending. E, Completed needle. Portion of cutting hair shown in figure 1, indicated at *a–b*.

placed on the slide and with a fine lip-pipette the surplus water is drawn off till only a thin layer is left, a little deeper than the diameter of the object. With the pipette a limited number of cells are placed in the middle of the drop. The needle is so held that the hand is relaxed and steadied by contact with the side of the stage. A considerable portion of the cutting hair is immersed entering the drop almost horizontally rather than at a sharp angle from above, to avoid film disturbances. With the hair, one of the cells is pushed near the front edge of the drop to a point where the decreasing depth of water slightly flattens and holds it so that it can be rolled over in any desired position. It may be advantageous to do the cutting in an outpocketing of the edge of the drop.

The cell is fragmented by pressure of the hair's rounded side, like a slender wire passing down through cheese, and not by a series of cutting strokes of the actual tip advancing into the object. This is the principle used by Chambers in some of his work where the terminal hair of a twice-bent needle, and not the tip itself, is used to fragment cells by pressing them either upward against the undersurface of an inverted cover-slip or downward against the inside of the surface film of the hanging drop ('18, figs. 7 and 8, pp. 133–134). The type of needle used in the hand method has a very flexible terminal region due to the very small size of the capillary and the hair's gradual elongation. This is quite different from needles used in the apparatus which have a coarser capillary and a very short stiff hair ('18, fig. 6, b, c, and d, p. 130). Owing to its flexibility, the hair can be so held that only its terminal region lies in contact with the slide, while behind this it slightly curves upward away from the slide, yet is still immersed in the drop. The cell to be cut is caught underneath the hair just behind the point where it loses contact with the slide. Thus the object is held between the hair and the slide, then by lowering the needle a trifle the cell is fragmented as the descending hair cuts it in two (fig. 1).

Surprising facility for making accurate dissections is soon acquired, and with this hand method a variety of operations is possible on cells as small as 25 μ in diameter. Objects can be completely fragmented or only partially severed or segments can be cut out. Nucleated fragments of Echinarachnius ranging in size from 10 × 20 μ and upward were easily cut from the eggs at the rate of about twenty-five in ten minutes. Another investigator used the method for cutting in large numbers a Holotrich protozoan, and it was also used to destroy blastomeres 25 μ in diameter in developing Nematode eggs.

After cutting, the fragments are pushed back into the deeper portion of the drop to avoid the surface-film pressure near its edges, and at the same time they are sorted to left and right. In some cases, such as eggs, fragments are difficult to distinguish from each other, and if they are kept separate, from the moment of cutting, much time is saved. Hence a number of cells can be cut in rapid succession and the fragments segregated in two groups as cut, to be picked up later at one time for experimentation. With a fine lip-pipette fresh cells are added to the drop or fragments removed without danger of mixing. This avoids the difficulties of working with an inverted cover-slip and its hanging drop which takes time and may cause mixing.

The capillary pipette used to pick up cells and fragments is more easily controlled by pressure from the mouth than by a hand bulb. Its end is flamed to soften sharp edges. In using such a pipette for minute objects it is desirable to have the bore as small as possible, which gives more accurate control of suction and causes less disturbance of the drop than would a larger bore. In many cases, however, living cells and fragments are injured by the pressure exerted upon them in passing up the capillary bore of too small a pipette, and it may be necessary to use one many times the diameter of the object, even though this is much clumsier to handle.

Chambers ('18) describes needle making, but the following suggestions are made, since needles for hand use differ

considerably from those made for the apparatus. Ordinary illuminating gas, rather than acetylene, is used for his described microburner. Soft glass tubing about 6 mm. in diameter fits the hand better than a smaller size. It is cut into lengths of about 20 cm., from each of which two needles are made as follows: In the middle of this tubing (fig. 2, A, *m*) intermediate tubing is drawn, in a bunsen burner, 2 to 3 mm. in diameter and about 5 cm. long. The original tubing is called primary shank, the intermediate tubing is called secondary shank. Overheating is avoided so that the taper between the two (fig. 2, B, *t* and *t'*) is short and at a sharp angle. The intermediate size is necessary, as fine capillary size is not readily drawn directly from heavy tubing. In the middle of the once-drawn tubing (fig. 2, B, *m'*) capillary tubing not over 0.2 mm. in diameter is drawn in a bunsen burner with the flame turned low. To attain this fineness it must be drawn quickly and to some length. Most of its middle region is destroyed, leaving a short length attached to the secondary shank of each of the two potential needles (fig. 2, C). In drawing tubing, whether coarse or fine, the glass is heated to the proper point in the flame, but is withdrawn from the flame just before the act of pulling.

In drawing the cutting hair from the capillary, the important factor is what Chambers calls the 'tug,' felt at the moment of separation. The primary shank of one of the twice-drawn needles is taken in one hand and its capillary in the other. It is so held that the part of the capillary about 3 cm. from the secondary shank (fig. 2, C, *m''*) is brought over the smallest possible flame of the microburner. It is allowed to descend slowly toward the flame, meanwhile the hands are steadily pulling away from each other to left and right at the same time. The capillary will melt just before it actually touches the flame and, due to the pulling, will separate just as it melts without becoming overheated; this results in the separation 'tug' of a good hair. The cutting hair at its extreme tip should not be thicker than 10 μ. In bending the needle an angle of 150° is made in the capillary

about 2 cm. from the secondary shank (fig. 2, E, *x*). If the angle is less, the hand must be held too low to secure relaxed contact with the microscope stage; if it is more, the hand must be held too high. The art of making needles for the hand method is very much simpler to master than that of making the more specialized needles for the apparatus, because the elongate flexible hair is much easier to draw than the short stiff type. Even though the tip of the needle breaks off when using, it immediately fills up with water and the remainder of the hair's cutting side surface is unaffected. If organic matter is allowed to dry on the needle the resulting roughened tip will tear the cells.

This method is not applicable to the more delicate apparatus operations and is not offered as a substitute for typical microdissection, but the very points that give the machine its special value for the most critical work, where it stands alone, make it too complicated and time-consuming for many simpler operations. It is possible to perform a wide variety of cell dissections with a simple, easily made, glass needle, held in the hand, cutting downward against the upper surface of an ordinary slide, seen through the uninverted image of a dissecting microscope, yet with remarkable accuracy.

ON THE QUESTION OF PNEUMATIC CAVITIES OF THE TEMPORAL BONE

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TWO FIGURES

The pneumatic cavities of the crania of mammals, according to Paulli(1), author of extensive researches in comparative anatomy, derive their morphological importance from the fact that by their development they give to the cranium of the species its characteristic form, which is molded in the process of adaptation.

The skull of man in regard to the pneumatization of the bones is distinctly inferior to those of other vertebrates. However, as regards the temporal bone with which we are now concerned, a number of observations are found in the literature describing increased pneumatization of various parts of the bone. These cases, pointing, as may be supposed, to a reversion to the organization of animals of a lower grade of development, receive a certain morphological significance, as well as a clinical importance in the possibility of diffusion of pus in cases of mastoiditis. We will describe below a case of increased pneumatization of the pyramids of the temporal bone which has come under our observation.

As is well known, the pneumatic cells of the temporal bone in man are concentrated mainly in the mastoid portion, where is also located the larger cavity, the mastoid antrum, which is surrounded by a number of small cavities, cellulae mastoideae, which open into it. The petrous portion has its own cells, cellulae petrosae, which Mouret(2), with reference to their position respective to the labyrinth, classifies as follows:

1) Supralabyrinthales; 2) sublabyrinthales; 3) occipito-jugulares. Moreover, in older individuals he has met three other groups: 1) Retrolabyrinthales; 2) praelabyrinthales, and, 3) supra-atticales.

Regarding cases of unusual pneumatization of this bone, we have found in the literature accessible to us the following observations: Ruffini(3) has described a temporal bone with a very rare anomaly: a peculiar swelling on the mastoid process with a cavity communicating with the mastoid cells. Ruffini believes this anomaly to be equivalent to a certain formation found in other mammals, particularly in carnivorous animals, and termed protuberantia mastoidea or bulla tympanica. A similar case of enlarged pneumatic cavities of the temporal bone has been observed by Carli(4), who holds, however, that homology with bulla tympanica is not justified, as this latter depends for its development on the os tympanicum, while the additional cavity under discussion develops together with the posterior section of the mastoid part. Kazzander(5) has described a temporal bone in which there were large pneumatic cavities in the squamous portion of the temporal bone. According to the same author, some others (Hirtl, Carli) have observed the extension of the pneumatic cavities of the mastoid process to the squamous portion of the occipital bone.

In our case the increase in pneumatization of the pyramids was so marked that the relief of the superoposterior surfaces was considerably modified.

The skull bearing this anomaly was that of a Jewish girl, aged 23, who died in the Odessa Municipal Psychiatric Hospital, where she had remained for seven months previous, with a diagnosis of dementia praecox. The body was brought to the Anatomical Institute of the University of Odessa, where the brain was removed on February 2, 1923, and the following results were obtained upon examination: The brain, together with the arachnoid and the pia mater, weighed 1265 grams; the granulations of Pacchioni were strongly developed; the brain showed no macroscopic devia-

tions from normal. The capacity of the skull estimated on the cadaver with water (Reichardt(6) was 1344 cc. An examination of the base of the skull from above revealed at once a singular appearance of the posterosuperior surfaces of the pyramids of the temporal bones. This singularity, as

2

Figures 1 and 2

can be seen in figure 1, is due to the fact that, first, each of these surfaces has an eminence in the shape of an arc with the convex side facing the posterior part of the skull. This eminence is larger on the right side. Further, they bear laterally from the internal acoustic meatus a number of cylindrical prominences running from the bottom and the

back upward and inward, and terminating in ovalospherical eminences on the border between the supero-anterior and superoposterior surfaces. The osseous tissue of the superoposterior surfaces of the pyramids did not show any signs of inflammation. The dura mater of the brain was normal.

Sections perpendicular to the axis of the right pyramid revealed the following: A section made 4 mm. from the edge of the internal acoustic meatus laterally (fig. 2) shows, from left to right, tympanic cavity, cochlea, internal acoustic meatus, and lastly, the peculiar air-containing cavities which have caused by their development the deviations in the shape of the superoposterior surfaces of the pyramids described above. The sections made medially from the internal acoustic meatus show that the apex of the pyramid also contains pneumatic cavities which surround the carotid canal. By means of an aperture of about 1.5 mm. diameter, these cavities join along the superior edge of the internal acoustic meatus the cavities lying on its outer side. A section made 1.5 cm. from the internal acoustic meatus laterally shows that the pneumatic cavities just described farther out have a connection with the petrous cells which, in the shape of a border 5 mm. wide, surround the region of the semicircular canals and in their turn coalesce with the mastoid cells. The same section shows that on the outer side of the mastoid antrum (which is of usual size—10 $\times$ 8 mm.) the pneumatic cavities extend to the part of the squamous portion which adjoins the pyramid, and, partly, to the root of the zygomatic process. No sections were made through the left pyramid, in view of the symmetry of the deformities and in order to preserve a half of the skull intact.

Among other peculiarities of the skull we will mention the fact that the digital impressions are rather deeper than usual as well as the impressions from the granulations of Pacchioni. The digital impressions are present not only on the base of the skull, but also on the internal surface of the frontal bone, where they extend nearly as far as the coronal suture. The internal surface of the squamous portion of the occipital bone

also bears very well-marked impressions of the gyri of the occipital portion of the brain. On the internal surface of the right parietal bone near the saggital suture is a groove for the granulations of Pacchioni, 1.5 × 1.2 cm.; the bottom of the groove is very thin, making it partly transparent to light; to the rear of this groove there are a few more fossae of smaller size. On the left parietal bone there are three fossae of circular shape about 1 cm. diameter. In conclusion, it must be noted that the grooves of the venous sinuses on the occipital bone, especially on the right side, are remarkable for their depth.

SUMMARY

On the skull of a twenty-three-year old girl the supero-posterior surfaces of the pyramids of the temporal bones presented unusual appearances: they protruded backward and inward and bore a number of eminences of cylindrical and ovalospherical shape. Sections perpendicular to the axis of the pyramid showed that these eminences were caused by pneumatic cavities extending from the apex of the pyramid to its base where they had a connection with the petrous and mastoid cells. Moreover, the skull had the marks of increased plasticity of the osseous tissue: comparatively deep digital impressions, grooves for the granulations of Pacchioni and for the venous sinuses. It is interesting to note that insanity was observed during life in this case (dementia praecox).

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A NOTE ON THE REACTIONS OF CHICK CHORIO-ALLANTOIS TO GRAFTING

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ONE PLATE (FOUR FIGURES)

In the course of work upon the grafting of portions of chick embryos on to the chorio-allantois of older embryos—work whose main results are being reported elsewhere—we encountered certain reactions of the host tissue which seem to merit separate record.

Previous workers on the subject have made a few observations. Minoura ('21) mentions an abnormal accumulation of leucocytes in the host tissue in successful grafts, and refers to the great growth of the stroma and to the fact that it is of somewhat looser texture than normal. He apparently has not encountered any reaction of the ectoderm or endoderm, since he specifically states that these remain one cell in thickness. Willier ('24) mentions that an intense proliferation of leucocytes occurs in the larger reaction masses only, and figures (his fig. 2) without description what appear to be hyperplasia and stratification of both ectoderm and endoderm. Danchakoff ('18) states that the chorio-allantoic ectoderm may become cornified, but gives no figures; she finds that the ectoderm in contact with the grafted tissue invariably shows abnormally numerous mitoses, and may proliferate in the form of irregular tongues or papillae toward or into the grafted tissue. She also mentions the great abundance of leucocytes in the stroma.

Our own observations are as follows:

GENERAL REACTION THICKENINGS

Very often the host tissue at the point of grafting is enormously thickened, from the normal membrane's three or four layers of cells (more in the neighborhood of large vessels) to a mass which may reach a millimeter in thickness. This is composed chiefly of a mesenchymatous tissue, often varying in density in different regions, and containing numerous vessels (figs. 1, 2). The outer epiblast in such cases has often undergone hyperplasia into a stratified epithelium.

These thickenings are frequently to be found in the total absence of living graft tissue, although they may coexist with what appear to be the dead remains of grafts (figs. 1, 4), which lie outside the membrane, and have apparently never 'taken.' For the present we cannot be sure whether a marked reaction thickening can coexist with a successful, living embryonic graft.

REACTIONS OF THE EXTERNAL EPIBLAST

a. Cornification

In such reaction thickenings the external epiblast may show not merely stratification, but cornification of a type similar to that seen in adult epidermis (fig. 2). Cornification may be present over one part of a thickening, absent over another, even though in the latter region there has been hyperplasia leading to stratification. Such cornification may extend beyond the central, markedly thickened portion of the reaction tissue on the surface of the surrounding comparatively thin region. We have not, however, succeeded in finding it upon chorio-allantois of normal thickness.

b. Formation of epithelial 'pearls'

In a number of reactions showing cornification there also exist epidermal structures which can best be described as epithelial pearls (figs. 1, 3, 4). These consist in most instances of spherical bodies composed of stratified epidermis surrounding a central core (occasionally double) of con-

centric layers of keratin. The basal layer of the epidermis is of course on the outside and usually consists of roughly cubical cells. The amount of central keratin varies considerably, as does the size of individual 'pearls.' These structures are occasionally found connected with the epidermis by means of epithelial stalks (fig. 4), or sometimes directly apposed to the external epidermis. No keratin or lumen has as yet been found in any of these stalks.

c. Other epidermal proliferations

In some areas numerous cells with all the appearance of detached epidermal cells are found among the mesenchymatous tissue, sometimes in strands, just below the exterior. Their appearance (fig. 4) seems definitely to indicate that there has occurred a rapid irregular proliferation of detached cells from the inner surface of the epidermis. In one case at least numerous strands consisting entirely of keratinized cells were found lying irregularly in the interior of a reaction thickening.

REACTIONS OF THE INTERNAL EPITHELIUM (ENDODERM) OF THE CHORIO-ALLANTOIS

This seems considerably less prone to hyperplastic changes, in the conditions of the experiment, than the epiblast. This, however, may of course be due to its being farther removed from the site of operative interference. When a reaction occurs in it, the cells of this epithelium become converted into a less flattened form and often undergo hyperplasia to form several layers. Occasionally a tendency also appears for the epithelium to become folded.

The above observations are perhaps of some interest as showing the great capacity of the tissues of the chick chorio-allantois to proliferate and differentiate in response to operative stimuli. One of us (P. D. F. M.) is continuing work with a view of investigating more closely the conditions on which proliferation depends. One remarkable fact is that the proliferation leading to the formation of epithelial pearls may be

markedly localized, pearls being absent from one part of a reaction thickening, abundant in another part.

The work was carried out with the aid of grants from the Department of Scientific and Industrial Research and from the Royal Society.

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PLATE 1

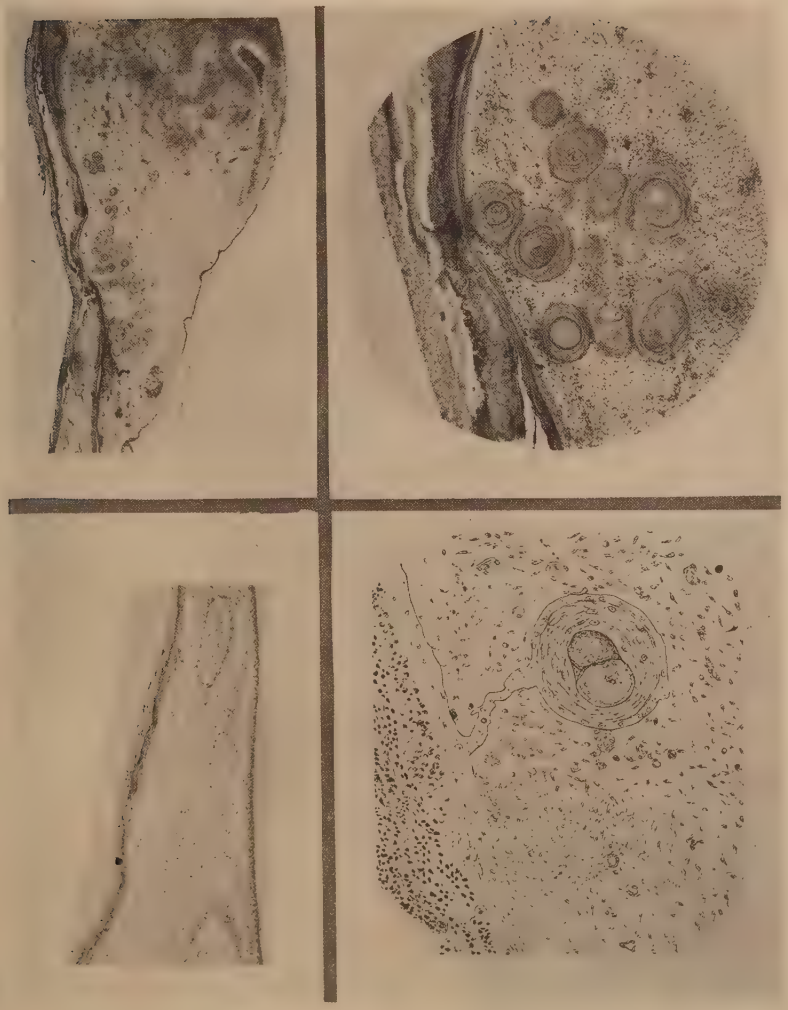
EXPLANATION OF FIGURES

1 Section through the major part of a large reaction thickening. At the left of the figure is seen the shell membrane; next, beyond a cleft-like space, the layer of keratinized ectoderm. In the thickened mesoderm groups of epithelial 'pearls' are seen. The limiting layer on the right is the endoderm. Between shell membrane and ectoderm, the mass, wedge-shaped in section, probably represents the degenerated graft. (Microphotograph, low power.)

2 Section through another reaction thickening, to show keratinization of the epidermis. (Microphotograph, medium power.)

3 Another section through the same reaction thickening as that in figure 1, to show epithelial pearls. One of these is in contact with the epidermis; another shows two keratin cores. (Microphotograph, higher power.)

4 Section through another reaction thickening, to show (above) a single epithelial 'pearl' with two keratin cores and stalk connecting it with the ectoderm, and (below) a mass of similar size representing an irruption of scattered epidermal cells into the mesenchyma. Blood vessels are numerous. External to the ectoderm is a mass (degenerated graft) similar to that shown in figure 1. (Camera-lucida drawing.)



1 3

2 4

AN ABNORMAL RELATION OF THE ILIAC ARTERY TO THE SCIATIC PLEXUS IN THE FROG

EDITH PINNEY

ONE FIGURE

Normally, in the frog the iliac arteries lie ventral to the tenth spinal nerve and dorsal to the nerves of the sciatic plexus. The accompanying figure shows a variation from this relation between the iliac artery and the sciatic plexus of the left side. Here the artery continues ventrally to the plexus from the tenth nerve, but soon turns and reaches its dorsal position by piercing the ninth nerve.

The arrangement here recalls the way in which the lateral head of the ulnar nerve pierces the medial head of the median nerve in man, although there the relation is between nerve and nerve, and not between nerve and blood vessel as in this instance.[1] Probably the developmental mechanics giving this result are the same in each case, being in man a normal occurrence, while the condition described here for the frog has not been observed before as far as the writer knows.

This frog was one of many used in class work in general zoology. Unfortunately, nothing was known of its behavior. The hind legs were normal and of equal size, and it seems likely that the blood supply of the left leg was in no way diminished on account of the peculiar course of the artery delivering it.

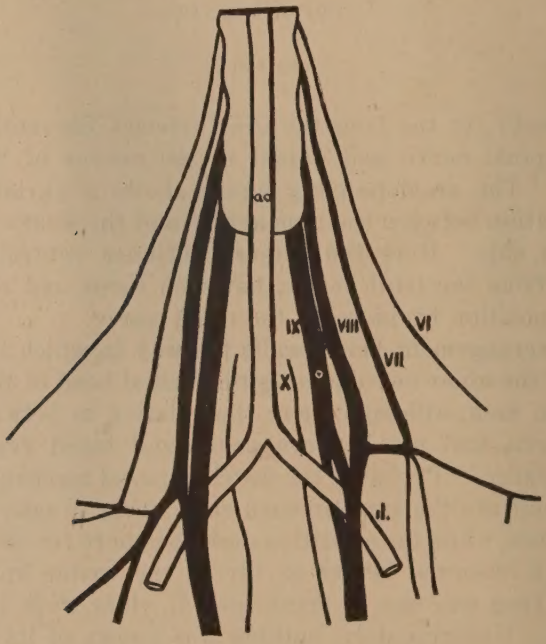
There is a slight difference in the formation of the sciatic plexus of the two sides, the left seventh nerve being about twice the size of the right seventh with consequent variation

[1] Abram T. Kerr, Brachial plexus of nerves in man. Am. Jour. Anat., vol. 23, p. 285.

in the size and distribution of their branches. Such differences are very common and it is doubtful whether it bears any relation whatever to the aberrant course of the iliac artery.

The case is reported in the hope that it may be of interest to students of nerve development.

Cold Spring Harbor, August, 1924.



BOOK REVIEW

ANATOMIE GÉNÉRALE ET TOPOGRAPHIQUE

There comes to my hands a new anatomy text-book (Anatomie Générale et Topographique) by Dr. H. Rouvière, professor in the University of Paris. It comprises three cloth-bound quarto volumes of 507, 504, and 664 pages, respectively, published by Masson & Company.

With accumulation of facts in so immense a field comes the time when exhaustive reference works must be separated from introductory texts for first-year medical students. Doctor Rouvière has written the latter type. He omits much of the less important details for the sake of writing a clear, simple description of each part. This policy has spared the reader the large amount of fine print so irritating to the chronically overstrained modern eye.

He makes a second splendid step in using the topographic method of treatment, discussing each organ as a unit, with adjacent muscles, blood vessels, lymphatics, nerves, etc., described close together. This is surely the ideal method for beginners. It allows them to form the essential topographic-image pictures. Students do not commonly take time to seek out descriptions of adjacent structures in the usual texts where the facts about a given area are separated among the various systems treated.

Throughout the text lie 988 figures, many in colors. They are well chosen and include many cross and longitudinal sectional diagrams. They do not try to show too much at once, and many are very good. They are placed adjacent to pertinent reading matter where they can be consulted without lost time. But the lines drawn in from the labels are apt to be confused with nerves and other intrinsic structures of the drawings. Use of dotted lines might have obviated this.

Serious injury to the value of the text outside of France has been done by use of colloquial French terms in place of the BNA, even in the figures where usage demands the Latin terms.

The format of the text is fine, the drawings and printed matter being most artistically arranged. The print is large, clear, and pleasant to read. But the paper is finished to a high gloss so that

the glare is hard on the eyes in bright light. Further, the paper is very fragile, so it tears with ease. This is an obnoxious trait in a much-used book. Anatomy texts are costly and should be worth keeping. They should be printed on paper of good quality and high tensility.

I am happy to commend all good traits in Professor Rouvière's text.

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